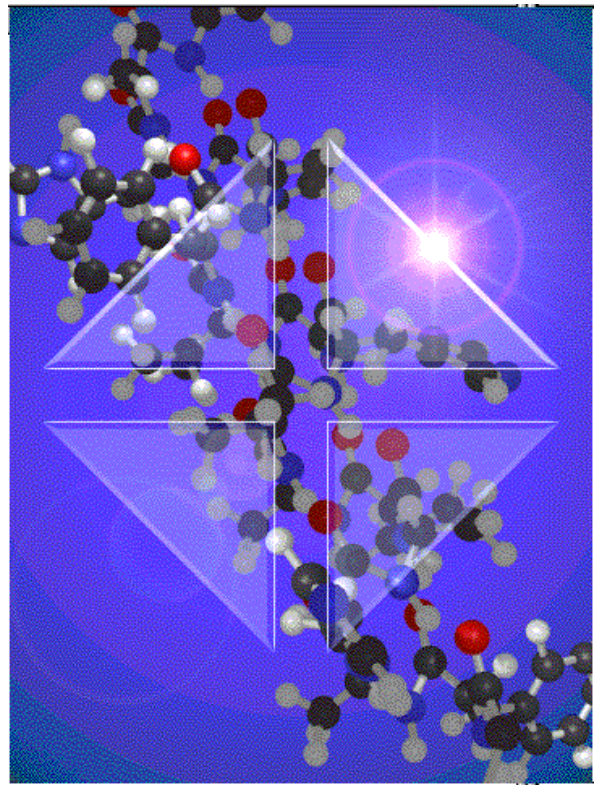
user manual
chromatography software

UNICORN Version 4.0

User Manual



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Preface

About this manual

This manual provides a reference to User functionality in UNICORN™ version 4.0 from Amersham Biosciences AB.
UNICORN is a complete package for control and supervision of chromatography systems, suitable for use with Amersham Biosciences' systems for the ÄKTA™ design platform (ÄKTA™ design systems), BioProcess™ System and BioPilot™ System. UNICORN consists of software which runs on an IBM-compatible PC under Microsoft Windows NT 4.00* or Microsoft Windows 2000, and hardware for interfacing the controlling PC to the chromatography liquid handling module.

The User manual is organised in 11 chapters and 4 appendices:

Introductory material	1. Introduction 2. UNICORN concepts 3. Logon and file handling
Methods and runs	4. Creating methods from method templates 5. Creating and editing methods 6. Performing a run 7. Scouting 8. MethodQueues
Evaluation	9. Presenting results 10. Evaluating results 11. Analysing results
Appendices	A. Strategy for ÄKTAdesign systems B. Evaluation functions and instructions C. Troubleshooting D. Mathematical models and statistics

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For the purposes of this user manual, examples are displayed for an ÄKTA*explorer* 100 system. You should refer to the user instructions specific to the chromatography system that you are using.

Assumptions

Two broad assumptions are made in this manual:

1. You should be familiar with the chromatography systems in your installation. Refer to the appropriate System Manuals for details.

2. You should be familiar with the general principles of using Microsoft Windows NT version 4.0 and/or Windows 2000 on your PC. Although UNICORN is a self-contained program package and does not require any direct interaction by the user with Windows, the user interface principles follow the conventions set by Windows programs.

   Many of the menu commands in UNICORN can be activated using the toolbar buttons, keyboard shortcuts and the shortcut menu. The availability of these command options is dependent on the active field or window in which you are currently working. The function of a toolbar button is displayed when you place the mouse pointer over a button. Shortcut menu commands are quickly found through use of the program.

Typographical conventions

Important! Note that UNICORN 4.0 is designed to run under English keyboard settings.

Menu commands, the names of dialogue boxes and windows, the contents of dialogue boxes windows, and option buttons are written with a **bold helvetica** typeface. Menu commands are written in the order of the menu name and then the command, separated by a colon. For example:

"Select **File:Save As** to display the **Save As** dialogue. Locate the destination drive and folder and enter a file name. Click on **Save**."

This directs you to click on the **File** menu and select the command, **Save As**. A dialogue called **Save As** is displayed in which you must locate the destination folder for the saved file and give the file a name. You then click on the button called **Save** in the dialogue to execute the save command.

A `typewriter-like` typeface is used for instructions as they appear in the text editor for methods and evaluation procedures. These are normally entered automatically by UNICORN.

Some menu commands also have shortcut keys on the keyboard, which are written within < > marks.

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1 Introduction

UNICORN is a control system developed and marketed by Amersham Biosciences AB for real-time control of chromatographic separation systems from a personal computer. The package operates together with systems in the ÄKTAdesign platform (referred to as ÄKTAdesign systems), BioPilot System and BioProcess System from Amersham Biosciences. Standard configurations are provided for ÄKTAdesign systems and BioPilot System, while configurations for process-scale separation with BioProcess System may be customised for a particular installation. UNICORN runs under the operating systems Windows NT workstation 4.0 and Windows 2000.

Functional features of UNICORN 4.0 include:

- One PC may control up to 4 separation systems directly.
- Network support allows up to 99 systems to be accessed from one PC.
- A Method Wizard, taking the user to step-by-step through the method development process.
- Method templates, providing method frameworks for most common applications, eliminating the need to program methods from scratch.
- Scouting facilities, allowing most parameters to be varied automatically in repeated runs to facilitate process development and optimisation.
- Dynamic graphical overview of active runs.
- Comparison of peak parameters from multiple runs.
- User-definable alarm and warning limits for monitor signals.
- Programmed sequential operation of multi-step separations.
- Batch operation and process documentation in accordance with the requirements of Good Manufacturing Practice (GMP) and Good Laboratory Practice (GLP).
- Comprehensive data evaluation software, including an Analysis module, enabling quantitation and molecular size determination.

In addition, UNICORN offers a comprehensive security system:

- Password control for all users, with access authorisation for other users' method and result files.
- Electronic signature handling, providing enhanced security for method and results files.
- Customised definition of access control levels.
- Audit trail for system operation.

Note: UNICORN must be correctly installed for stand-alone or network operation before the software can be used. Network considerations, software installation and administration of system and user definitions are described in the UNICORN 4.0 Administrative and Technical Manual.

2 UNICORN concepts

This chapter introduces the basic concepts that are specific to UNICORN. For a description of how to work with the Windows NT or Windows 2000 operating system, see your Windows system documentation.

Material in this chapter is divided into 9 sections, dealing with:

- UNICORN control software
- UNICORN user interface
- Files and folders
- Methods and method structure
- System Control
- Scouting
- Evaluation
- Network considerations
- Security and administration

2.1 UNICORN control software

UNICORN runs under the Windows NT and Windows 2000 operating systems, and provides facilities for method-controlled operation of chromatography systems as well as real-time monitoring and subsequent evaluation of the separation process.

2.1.1 Strategies

Part of UNICORN software (referred to as the strategy) is system specific. The strategy defines what is available in method and manual instructions, system settings, run data, curves and method templates. Most of this manual describes the user interface in UNICORN independent of the strategy. Strategy-dependent instructions are listed in Appendix B.

2.2 UNICORN user interface

2.2.1 Toolbar Guide

The UNICORN Toolbar Guide dialogue is shown after start-up and logon and provides an introduction to the Main Menu toolbar buttons.

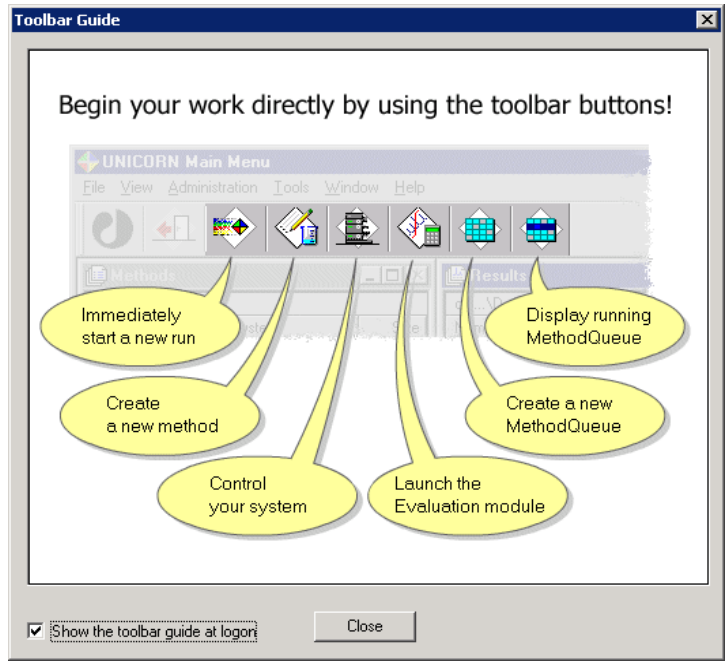

Toolbar Guide
Begin your work directly by using the toolbar buttons!
UNICORN Main Menu
File View Administration Tools Window Help
Immediately start a new run
Create a new method
Control your system
Launch the Evaluation module
Create a new MethodQueue
Display running MethodQueue
Show the toolbar guide at logon
Close

Fig 2-1. UNICORN Toolbar Guide dialogue.

The Main Menu toolbar icons allow you to begin using UNICORN quickly, for example, to create a new method in the Method Editor, start an instant run, open a result file for evaluation, or execute manual instructions in System Control (see Section 3.2).

2.2.2 Software modules

UNICORN control software consists of four integrated modules:

- The **Main Menu**, with functions for file handling and administrative routines such as definition of available chromatography systems and maintenance of user profiles.
- The **Method Editor**, where methods for pre-programmed control of chromatography systems are created and edited.
- The **System Control** module, which permits manual or method-based control of chromatography systems and on-line monitoring of separation processes. There may be up to four independent system control modules on one computer, for controlling up to four separate systems.

- The **Evaluation** module, with extensive facilities for presenting and evaluating stored results from separation processes.

These modules are present on the Windows taskbar.

To minimize a module to the taskbar, click on the Minimize button at the right-hand end of the window title bar. To minimize the whole of UNICORN click on the <Windows + M> keys on the keyboard.

Note: Minimizing a module window to the taskbar does not close the module. Once opened, UNICORN modules remain active until you quit the program. A minimised System Control module may thus be actively in control of a running process.

2.2.3 On-line help

A comprehensive on-line help utility is included in UNICORN software. Entry to the general help utility can be accessed from the **Help** menu. Dialogue- or window-specific help topics can be obtained by clicking on the **Help** button in the dialogue or by pressing <F1> on the keyboard. In the dialogues for method instructions, procedure instructions and system settings, pressing <F1> when an instruction is highlighted will display an information box with short help on the function and use of the selected instruction.

2.3 Files and folders

UNICORN Main Menu interface divides user files into two categories, for methods and results. Only folders to which the current user has access are shown in the Main Menu windows, **Method** window and **Results** window. Files may be displayed in several viewing options (see Chapter 3 for more details).

2.3.1 Method files

Method files contain instructions for controlling a run and are shown in the **Methods** window of the Main Menu.

The **Methods** window also displays icons for MethodQueues, which allow several methods to be run in an automatic pre-programmed sequence on the same or different systems.

2.3.2 Result files

Result files are created by UNICORN when a method is run and contain:

- Run data from the monitors in the chromatography system (e.g. UV absorbance, flow rate, conductivity etc.).

- Saved results from evaluation of the run data (see Chapter 10).
- Run documentation including information on, for example, the Logbook, calibration settings, scouting parameters, text method etc.

2.4 Methods

Chromatography runs are programmed as *methods* in UNICORN. This section gives a brief overview of the concepts and principles of methods. See Chapters 4 and 5 for a description of how to program methods, and Chapter 6 for how methods are used to control chromatography systems.

2.4.1 Method creation

Methods can be developed in a number of ways including the Method Wizard (see "Method Wizard" on page 33), by using an existing method template (see "Template-based method creation" on page 34), or by using the Method Editor (see "Method Editor" on page 36).

The Method Wizard takes the user step-by-step through the method creation process. Method templates are basic methods which provide convenient starting points for developing customised methods. Creating methods with the Method Editor is best-suited for methods that require many fine-scale modifications.

Method Wizards or templates for most chromatography techniques are supplied with UNICORN installations for ÄKTAdesign systems. New methods are created by selecting a suitable system, technique, template and column. By selecting a column, recommended flow rate, column volume and pressure limit for the selected column are copied into the method. The method can then, if necessary, be modified on the Variables tab or in the Text instructions. Fully adequate customised methods for many applications can be created simply by adjusting the values of method variables in a suitable template.

2.4.2 Method structure

Blocks

Methods in UNICORN are usually divided into blocks. Blocks typically represent well-defined steps in the separation procedure, such as:

- column equilibration
- sample application
- elution
- column wash

Blocks are also used for operations which are to be performed conditionally (e.g. starting fraction collection if a UV-absorbance peak is detected). The user determines the number of blocks in a method, as well as the names and functions of the blocks.

Method base

Method blocks are written in one of three bases, which defines the unit for the breakpoints in the block:

- time (min)
- volume (ml or l according to the strategy)
- column volume (set by the user)

Different blocks in the same method may be written on different bases: for example a column wash block might be written in terms of column volumes while sample loading might be more appropriate in absolute volume.

Instructions

Each block in a method consists of a series of instructions that request specific operations in the system. For example:

```
Flow 8.0 {ml/min}
```

sets flow rate for the pump to 8.0 ml/min.

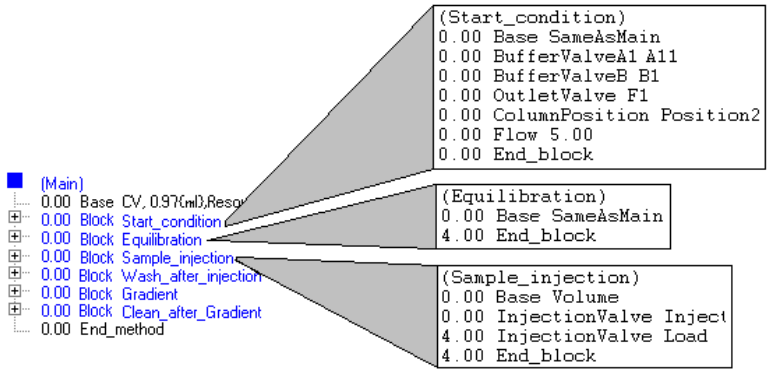


Fig 2-2. Relationship between blocks and instructions. The method (left) is written as a series of blocks, each of which consists of instructions for performing one or more specified tasks (right).

Breakpoints

Each instruction in a method block is issued at a specified breakpoint according to the method base. The first instruction in a block is always at breakpoint 0, and all other breakpoints are counted from this point. For example, the instruction

```
5.00 Flow   8.0
```

will set the flow rate to 8.0 ml/min at 5 units (minutes, ml, l or column volumes according to the method base) after the start of the block.

Method variables

Breakpoint values and instruction parameters may be defined as variables. This is a powerful facility for:

- constructing a "framework" method with default parameter values which may easily be changed either to create variants of the same method or to adjust the parameter values at the start of a run (see Section 4.1).
- constructing a method for use in automatic method scouting, where one or a few parameters are varied systematically (see below and Chapter 7).

Using variables makes it easy to adapt a method to a particular chromatography run. For example, in the block below, the breakpoint for switching the InjectionValve from Inject to Load is defined as a variable with the name `Empty_loop_with`. The value supplied for this variable when the method is run will set the volume with which the loop is emptied:

```
(Sample_Injection)
0.00  Base Volume
0.00  InjectionValve Inject
(4.00)#Empty_loop_with InjectionValve Load
4.00  End_Block
```

Variable names in method instructions are always preceded by the # character. The value for the variable is shown in parentheses in the instruction (in the example above, `4.00` is the value for the variable `Empty_loop_with`).

By using variables, a method may be displayed either in detail as Text instructions or in a condensed form as variable values in Run Set-up mode. This is illustrated in Figure 2-3. The Run Set-up mode is displayed when the method is run, allowing variable values to be set at the beginning of the run. Up to 500 variables can be included in a single method.

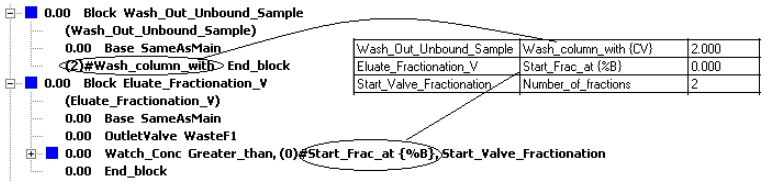


Fig 2-3. Relationship between variables in Text instructions and in the Variables tab of run set-up.

2.4.3 BufferPrep

BufferPrep is available for some ÄKTAdesign systems. BufferPrep allows a buffer of defined pH and salt concentration to be prepared on-line from four stock solutions. BufferPrep should be used for ion exchange chromatography.

2.5 Scouting

Scouting is a powerful facility for repeating a method run automatically with predetermined changes in values for one or more variables in the method. Typically, scouting is used for optimising chromatographic processes or monitoring column performance, e.g. by determining the dependence of resolution on flow rate or gradient slope.

A scouting scheme in UNICORN is defined as part of the method. Values for the scouting variables may be set in the method and/or adjusted when the method is started. The following scheme for example scouts for flow rate while holding pH constant:

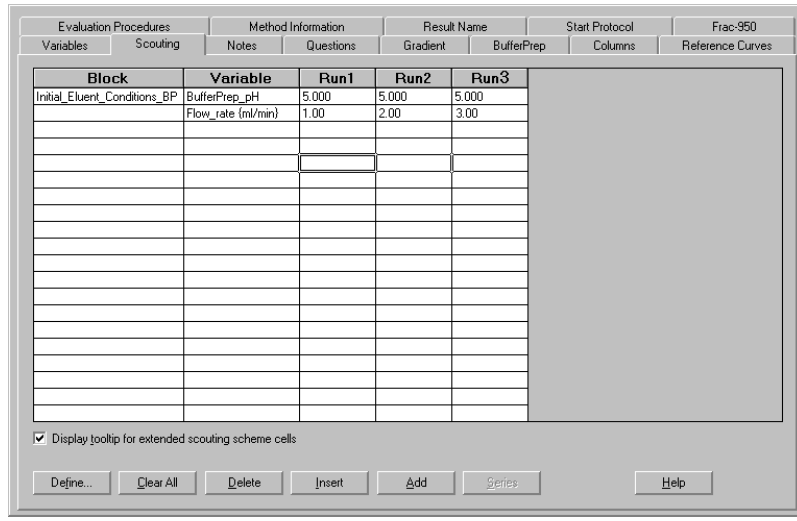


Fig 2-4. Scouting scheme for testing flow rate while holding pH constant.

2.6 System Control

2.6.1 Control facilities

The system control module allows independent control of up to four chromatography systems from one computer, with continuous real-time monitoring of the separation process. The run status can be displayed as:

- numerical display of run data from selected monitors
- graphical display of curves from monitors
- a flow scheme showing the current open flow path in the system
- a logbook recording the control events in the run.

Systems can be controlled either manually with interactive commands or through pre-programmed methods.

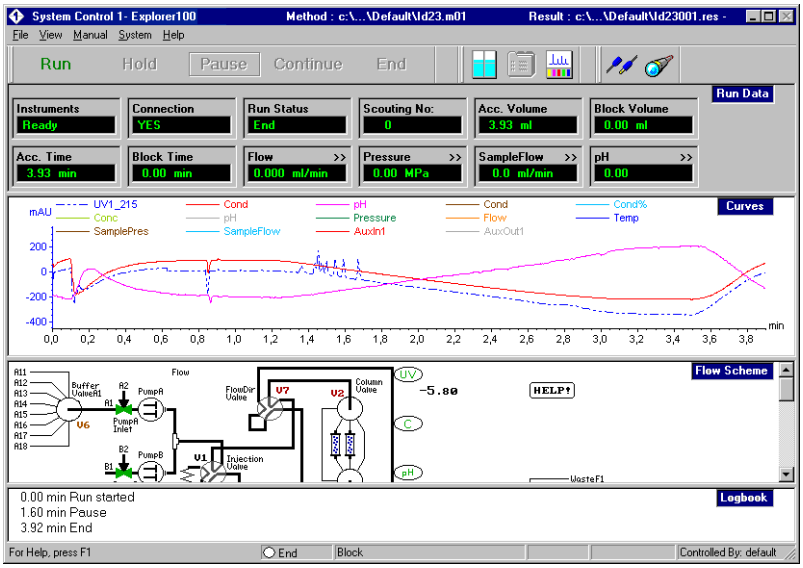


Fig 2-5. The system control screen.

By using MethodQueue facilities, several methods may be run in a predefined automatic sequence involving one or more chromatography systems. With suitable chromatography system equipment, this allows unattended operation of quite complex multi-step separation processes.

2.6.2 System connections

For controlling a separation process, the operator establishes a *connection* between the computer and the chromatography system in one of the system control windows on UNICORN desktop. Two kind of connections may be established:

- *Control mode connections* which permit full control of the connected system.
- *View mode connections* from which the progress of the separation can be monitored but the system cannot be controlled.

A system can be started from a computer in, for example, the laboratory. Control of the system can be released without affecting the run and the control of the system can be later taken from another computer station, for example, in the office.

Each chromatography system can have only *one* control mode connection at any one time, but it can have *several* view mode connections. In a network installation, the same or different users may establish simultaneous view mode connections to one system on different computers. This allows a running process to be monitored from several locations at the same time.

2.7 Evaluation

The Evaluation module (chapter 9 and 10) provides extensive facilities for presentation and evaluation of separation results. Essential features of evaluation include:

- *Curve manipulation*. A wide range of operations can be performed on curves, such as addition and subtraction of two curves, differentiation, integration, normalization and scaling. The original raw data curves are always kept unmodified in the result file.
- *Curve comparisons*. Curves from different result files can easily be compared in the evaluation module.
- *Peak purity and peak identification*. Ratios between UV curves measured at different wavelengths give useful information about peak purity or peak identity.
- *Peak integration*. Peak area calculations are performed with respect to a baseline either set by the operator or calculated by the software. Peak data are presented in a tabular form.
- *Fraction histograms*. If the result file contains fraction marks, a curve can be converted automatically to a histogram for collected fractions.
- *Evaluation procedures*. Operations performed in the evaluation module can be recorded as an evaluation procedure and repeated for other result files with a single menu command. Evaluation procedures may be executed either automatically on completion of a method run or interactively from within the evaluation module.
- *Reports*. Comprehensive reports of the evaluation results can be generated for hard-copy documentation of the separation process. Generation and printing of reports may be included as an operation in an evaluation procedure to automate process evaluation and documentation.

2.8 Network considerations

UNICORN can be installed on a stand-alone PC workstation or PC workstations in a network.

2.8.1 Stand-alone installation

In a stand-alone installation, up to four chromatography systems may be physically connected to and controlled from the workstation where UNICORN is installed.

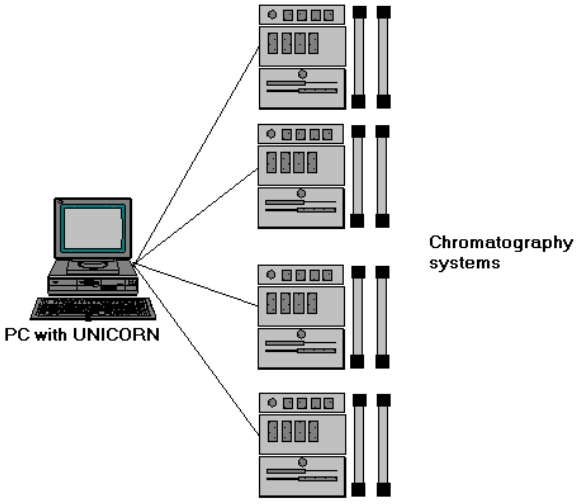


Fig 2-6. Stand-alone installation of UNICORN on a workstation, which can control up to four separate chromatography systems.

2.8.2 Network control from a remote workstation

In a network installation, each chromatography system is physically connected to a workstation, but may be controlled from any workstation in the network on which the UNICORN software is installed. A workstation to which a system is physically connected is referred to as a local station. Other workstations in a network installation are called remote stations.

During installation of UNICORN for the first time on a workstation in a network configuration, certain files are copied to the network server. These files include UNICORN user files and strategy files, and are the global settings for all UNICORN users in the network (see Chapter 3 in the UNICORN 4.0 Administration and Technical manual). UNICORN program files and templates are locally installed on each workstation in the network, and the network is used as the medium of communication to establish control with the chromatography systems.

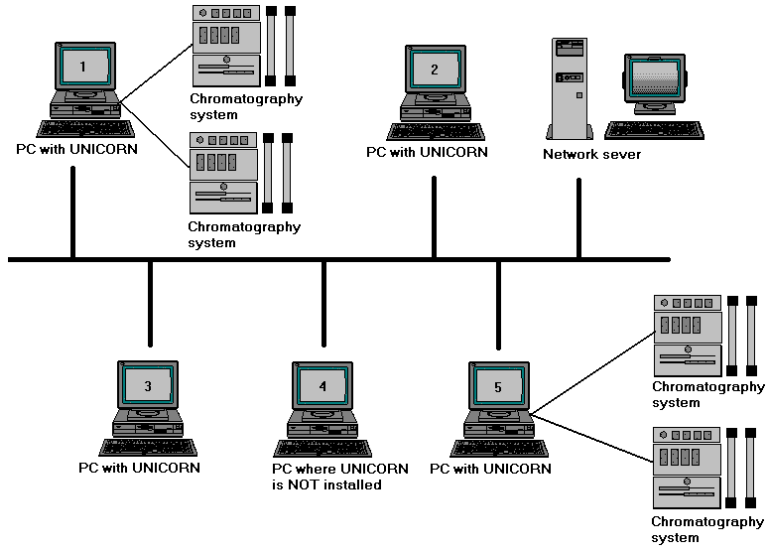


Fig 2-7. A network installation with 4 chromatography systems and 5 workstations (PCs). The chromatography systems physically connected to PCs 1 and 5 can be controlled locally. Alternatively, any of the PCs with UNICORN installed can be used to remotely control any of the chromatography systems via the network. In this example, PC 4 is connected to the network but it cannot be used to control any chromatography systems since it does not have UNICORN installed. Note also that the server does not have UNICORN program files installed and is not involved in the control process *per se*.

2.9 Security

Security features in UNICORN include:

- *Access security*. Use of UNICORN is restricted to authorised users. Each user is assigned an access level which defines the functions that the user is permitted to use.
- *Connection security*. Running systems may only be controlled from one connection. Systems may be locked with a password to prevent other users from changing run parameters.
- *Data security*. Result files can be saved automatically at pre-set intervals during a run to minimise data loss in the event of system failure. In a network installation, results are saved on the local station if network communication fails.

- *Electronic signatures*. Method and result files can be electronically signed, thereby providing enhanced file security.

Security features, network and administrative aspects are discussed in more detail in the UNICORN 4.0 Administration and Technical manual.

3 Logon and file handling

3.1 Logging on

When you start the computer you must log on to Windows NT or Windows 2000 before you can log on to UNICORN and begin working.

1. To start UNICORN, locate the program in the Windows **Start** button under **Programs:Unicorn:UNICORN4.00**. Alternatively, double click on the UNICORN icon on the desktop if this option was selected during installation.

If UNICORN is already started and the previous user has logged off, click on the **Tools:Logon** menu command or click on **Logon/ Logoff** icon in the Main Menu module.

Fig 3-1. The Logon dialogue.

Click on your username in the list and type your password. The first time you log on to the system as a user, you are given the option of changing your default password ("default"). Click on the **OK** button to log on. If you cannot remember your password, you cannot log on to UNICORN. Ask your system administrator or other user with sufficient authorisation to give you a new password.

Note: If UNICORN has been installed so that no password is required for logon, you need only select you username and click on **OK** to proceed.

Press the **Cancel** button to abandon the logon attempt.

3.2 Toolbar Guide

The UNICORN Toolbar Guide dialogue is shown after start-up and provides an introduction to the Main Menu toolbar icons.

The Main Menu toolbar icons allow you to begin using UNICORN quickly, for example, to create a new method in the Method Editor, start an instant run, open a result file for evaluation, or execute manual instructions in System Control.

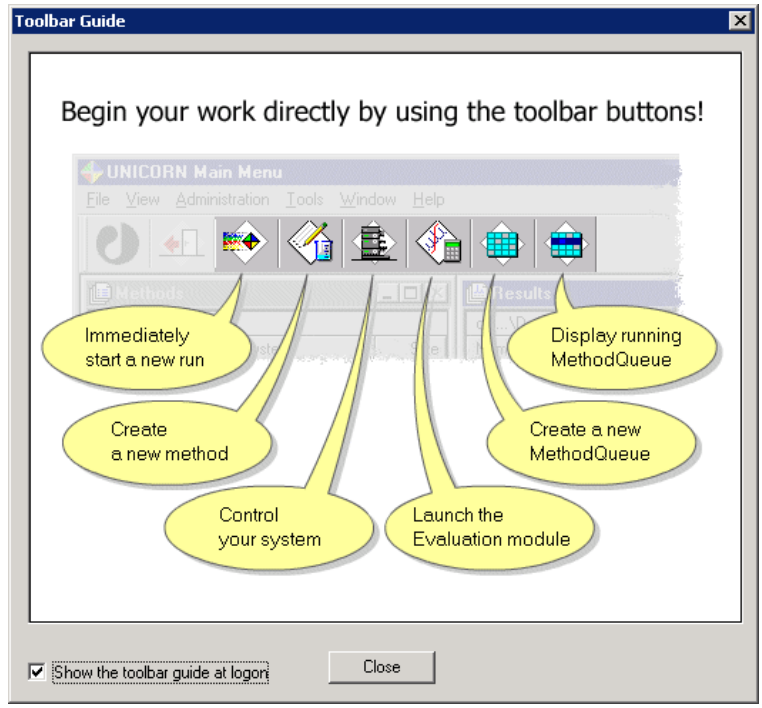

Toolbar Guide
Begin your work directly by using the toolbar buttons!
UNICORN Main Menu
File View Administration Tools Window Help
Immediately start a new run
Create a new method
Control your system
Launch the Evaluation module
Create a new MethodQueue
Display running MethodQueue
Show the toolbar guide at logon
Close

Fig 3-2. UNICORN Toolbar Guide dialogue.

The toolbar icons are:

About UNICORN — This gives you information about the UNICORN version installed, copyright and web address for obtaining more information.

Logon/Logoff — This allows you to log on or off UNICORN as appropriate.

Instant Run

This opens the **Instant Run** dialogue (Fig. 3-3) in which you can select the system (Wizard or template) to run. Press on the **Run** button to view the Start protocol and to start the run (template), or view the Wizard.

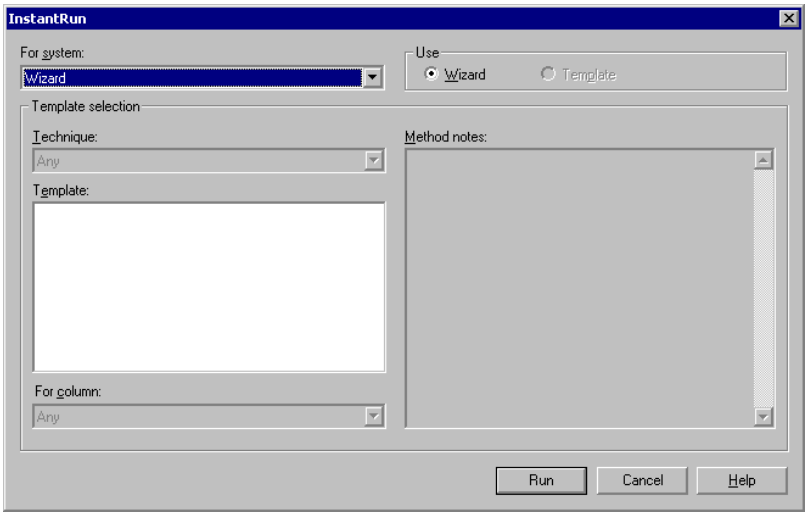


Fig 3-3. The Instant Run dialogue.

Note: Use of this function requires that Wizards or templates are defined. Standard systems are supplied with Wizards and templates, but custom systems require that the user makes templates.

New Method

This immediately starts the Method Editor module and displays the **New Method** dialogue (see "Creating a new method" on page 33).

System Control

This activates the first connected System Control and displays the **Manual instruction** dialogue (see "Manual instructions" on page 160).

Evaluation

This displays the **Open Result** dialogue. Select a result file and click on **OK** to launch the Evaluation module (see "Presenting results" on page 185).

MethodQueue Prompts the **MethodQueue Editor** dialogue (see "Editing MethodQueues" on page 181).

Display Running... Prompts the Running MethodQueue dialogue (see "Displaying MethodQueues" on page 182).

3.3 UNICORN Main Menu windows

The two Main Menu windows display the folders to which you have access within UNICORN and the method and result files within the currently open folder respectively. You can only see method files written for systems to which you have access.

3.3.1 Creating a new folder

To create a new user-specific folder:

1. Select the appropriate window, **Methods** or **Results**, in which you want to create a new folder.
2. Select **File:New:Folder** or **New Folder** from the shortcut menu. The **Create New Folder** dialogue is displayed.
3. Enter the name of the new folder and click on **OK**. The new folder is displayed in the appropriate window. Any user that has access set to the main folder in which the new folder was created also has access to the folders and files contained therein.

3.3.2 Opening and running method files

To open and edit a method file in the Method Editor click on a file in the **Methods** window and select **File:Open**, or click on the file with the right mouse button and select **Open** from the shortcut menu. Alternatively, double click on a file to open it.

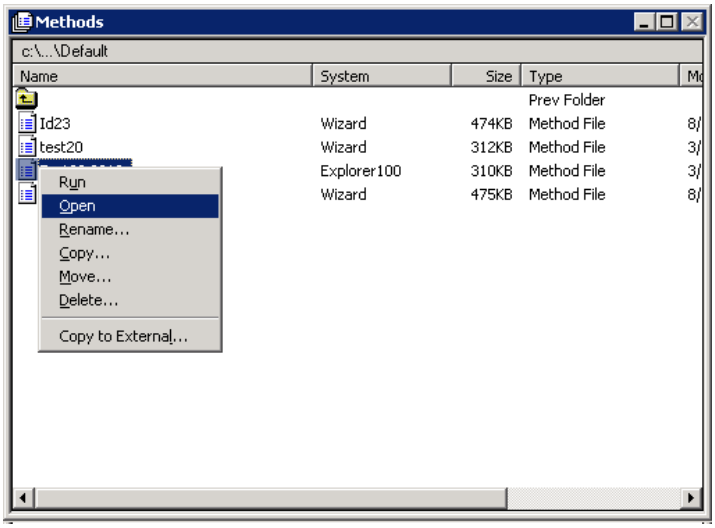


Fig 3-4. The Method window with shortcut menu selected.

Method files can be run directly in the System Control module. Alternatively, click on a file in the Main Menu **Methods** window and select **File:Run**, or click on **Run** in the shortcut menu.

3.3.3 Opening result files and Quick View

Result files located in the **Result** window can be opened in the same way as method files (see Section 3.3.2). A result file is opened in the Evaluation module (see Chapters 9 and 10).

It is also possible to preview the first UV curve in the first chromatogram for a selected result file without opening it in the Evaluation module. This facilitates selection of the correct result file for opening.

1. Select the result file(s) to be opened and select **Quick View** from the shortcut menu.

   The **Quick View** dialogue is displayed containing the chromatogram with the first UV curve displayed.

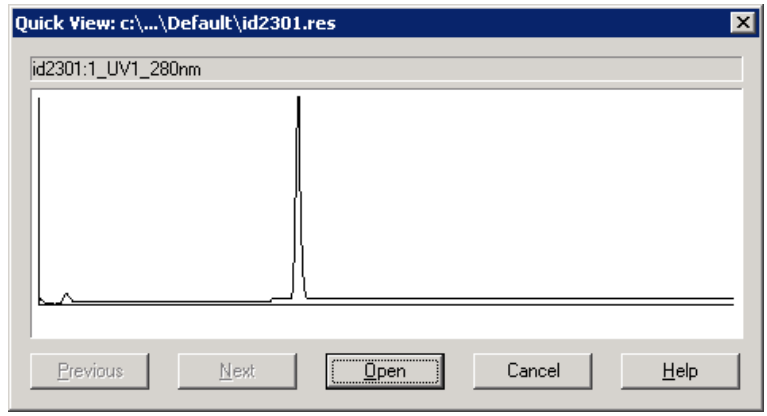


Fig 3-5. Quick View dialogue.

2. If you choose to view several result files, use the **Next** and **Previous** buttons to move between them.

3. To open a result file in the Evaluation module directly from the **Quick View** dialogue click on **Open**.

4. Click on **Cancel** to close the dialogue.

3.3.4 Presenting files

The way files are presented in the windows can be set from the **File** menu or from the shortcut menu. Presentation options are:

- view mode
- sorting order
- filter (for displaying only a chosen set of objects, e.g. methods for one system)

View mode

From the **View:Show** menu or from the **View** options in the shortcut menu you can select to display the contents of the windows in several Windows based alternatives. View the files either as a details list (**View:Details**), a simple list (**View:List**), large icons (**View:Large Icons**) or small icons (**View:Small Icons**).

The details list includes a small icon identifying the type of object, file name, file type, the last modified date and time, and the date and time of file creation.

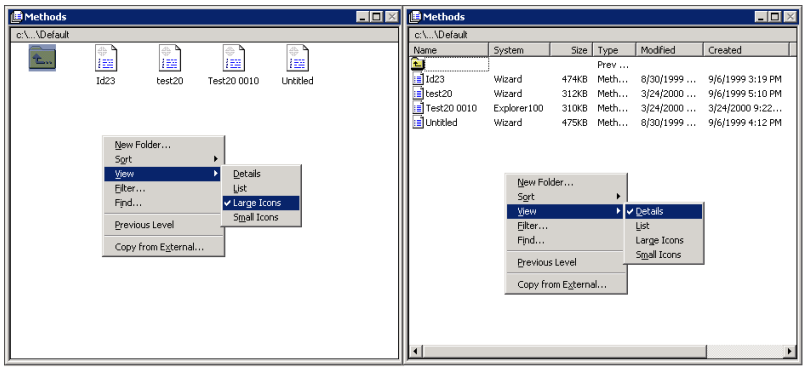


Fig 3-6. Large icon (left) and detail (right) display modes illustrated for the Methods window.

Sorting order

In the details list viewing mode, files can be sorted in the window according to one of the following:

Name	alphabetical order or reverse alphabetical order
System	alphabetical order or reverse alphabetical order (**Method** window only)
Size	smallest or largest files first
Type	alphabetical order of file extension type
Modified	last recently modified files first
Created	most recent creation dates first

To change the sorting order, choose **Sort** from the shortcut menu or **File:Sort**, and choose the appropriate sorting order from the menu cascade. Alternatively, click on the column headers in the window for **Name**, **System**, **Size, Type, Modified** and **Created** to toggle file sorting accordingly. Click a second time on the same sorting option and the files are sorted in reverse alphabetical order, increasing file size etc. as appropriate to the selection. Changing the sorting order affects only the currently active window.

Filter

To restrict the files displayed according to file name or the system (in the Methods window only) with which they are associated, choose **Filter** from the shortcut menu or select **View:Filter**. Mark the system(s) for which you want to display files, and enter a file name specification if required. Click on **OK** to activate the filter. The filter only affects the display in the **Methods** window.

You can use standard Windows wildcard characters in the file name specification (* stands for any number of characters, ? for any single character). For example:

iex	will display only files named iex
iex*	will display all files with names beginning with iex
*iex	will display all files with names ending with iex
?iex	will display only 4-character names ending with iex

If a filter is active, this is indicated in the title bar of the panel (e.g. c:\...\Default [Filter on system]). To display all files, choose **Filter** and click on all of the available system check boxes.

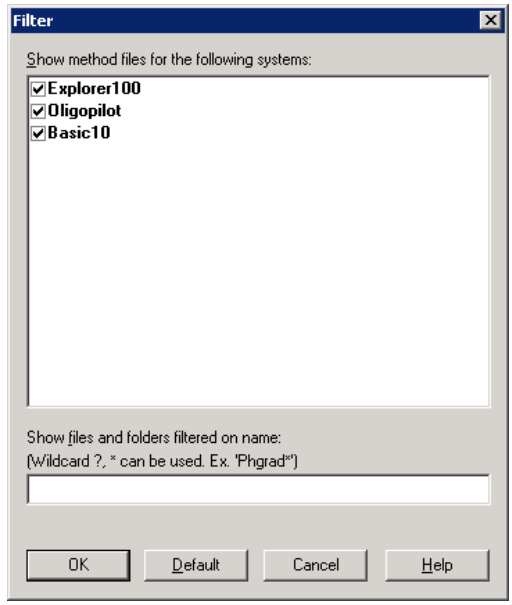


Fig 3-7. The Filter dialogue.

3.3.5 Finding files

To find a file:

1. Choose **Find** from the shortcut menu or select **File:Find**. In the displayed **Find files** dialogue, enter a file name specification in the **Search for files filtered on name** field. You can use standard Windows wildcard characters in the file name specification (see above under Filter).

2. You can restrict the search further if required:

   - Enter a name in the **Named** field.
   - Choose a file type from the pull-down menu for **Type** (**All**, **Folders**, **Method files** or **MethodQueue files** for the **Methods** window; **All**, **Folders**, **Result files** or **Scouting files** for the **Results** window).
   - Use the **Question** and **Answer** fields to enter text strings to identify any desired **Question** and **Answer** text identifiers.
   - Click in the **Date** box and set the date limits for the search.
   - Check **Include subfolders** to search through all the folders to which you have access. If **Include subfolders** is not checked, the search will not access subfolders.

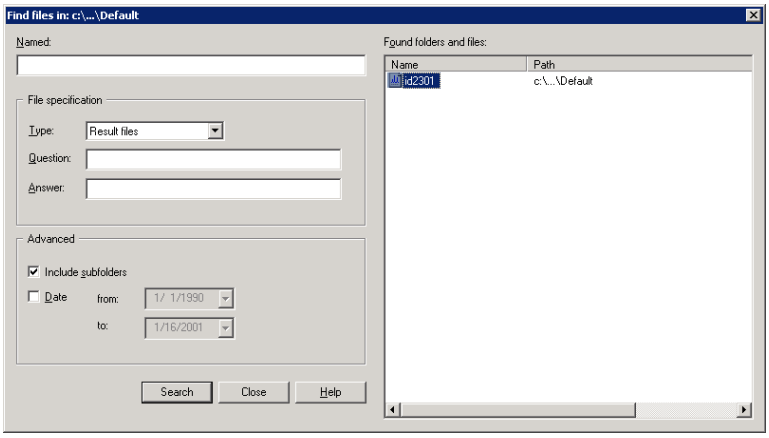


Fig 3-8. The Find file dialogue.

3. When you have entered all parameters, click on **Search**. The result of the search is shown in the **Found folders and files** field.

4. Double-click on a file in this list to return to the Main Menu with the selected file highlighted in the appropriate window. If you click on **Close** (with or without selecting a file), you will return to the Main Menu with the window display unchanged.

3.3.6 Copying and moving files and folders

You can copy and move files and folders to another folder that is specific to your user name. You can also copy or move files to and from an external drive and folders available on the network. If you copy or move a folder, all files within the folder will also be copied or moved.

Copying or moving files and folders

1. Select one or more files or folders in either the **Methods** or **Results** window of the Main Menu. To select multiple files or folders, use the standard Windows function keys <Ctrl> or >Shift>.

2. Click with the right mouse button on any file/folder icon and choose the **Copy** or **Move** command from the shortcut menu, or select **File:Copy** or **File:Move**. The **Copy** or **Move** dialogue is displayed respectively.

3. Select an available folder or the diskette drive to which you want to copy or move the file/folder and click on **OK**. Copied files and folders are user-specific.

Note 1: You cannot copy or move files between the **Methods** and **Results** windows of the Main Menu.

Note 2: Explicit authorisation is required to copy or move files (see Chapter 4 in the UNICORN 4.0 Administration and Technical manual).

Note 3: To copy a file within the same folder, open the file in the relevant UNICORN module, e.g. a method file in Method Editor or a result file in the Evaluation module, and use the **File:Save As** command in the module to save the file with a different name from the original.

Note 4: When copying to a diskette (a:) use **Copy to External** so that the files are automatically compressed.

Note 5: If you are moving a method to another system, you must always use the **Copy to External/Copy from External** functions. This will give you the possibility of connecting the method to the appropriate system. The extension for the method file name is used to identify the system for which the method has been created. An incorrect extension may result in syntax errors in the method or the method not being visible in the **Methods** window of Main Menu.

Copying files to external

Copying files to external may be useful when you want to store all results, documentation etc. in a common project folder on the network, or want to back up the files in a special place.

To copy a method file, scouting method folder or result file to external:

1. Select the file to be copied in either the **Methods** window or **Results** window.

2. Select **Copy to External** from the shortcut menu or select **File:Copy to External**. The **Copy to External** dialogue is displayed.

3. Select the destination drive and folder and click on the **Save** button.

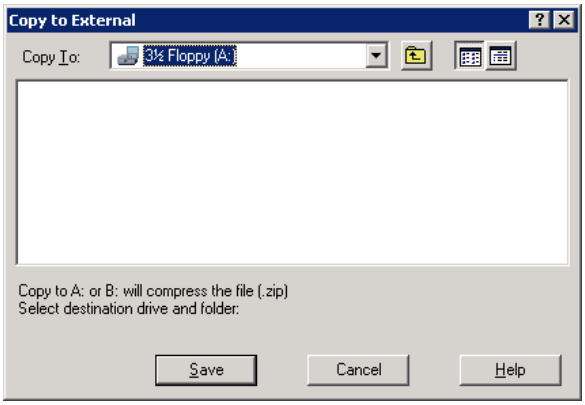


Fig 3-9. The Copy to External dialogue.

Note: If you select the 3½" Floppy Drive (a:) as the destination drive, the files will be automatically compressed into a .zip file thus allowing approximately 5-10 times the storage capacity. Moreover, if the zipped file is greater than the storage capacity of the disk, the file saving is automatically spanned across several disks. Files are automatically decompressed when using the **Copy from External** operation (see below). The zip function does not work if you select the **Copy** function.

Copy files from external

Method and result files can be copied from external. If the selected files have been compressed using the **Copy to External** function, then these will be automatically decompressed. To copy a method or result file from external:

1. Select the destination folder in the **Methods** window or **Results** window.

2. Without selecting a file icon, bring up the shortcut menu and select **Copy from External** or select **File:Copy from External**. The **Copy from External** dialogue is displayed.

4. Select the desired file(s) from the relevant source drive and folder. Click on the **Save** button.

5. If result file(s) were selected, these will be copied into the previously designated folder in the **Results** window.

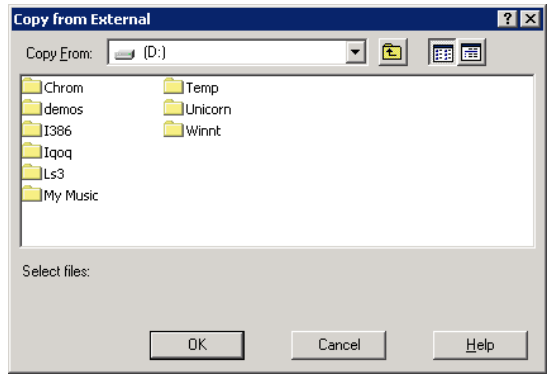


Fig 3-10. Copy from External dialogue, in this example, used to copy method files.

6. If method file(s) were selected, the **Method-System Connection** dialogue is displayed.

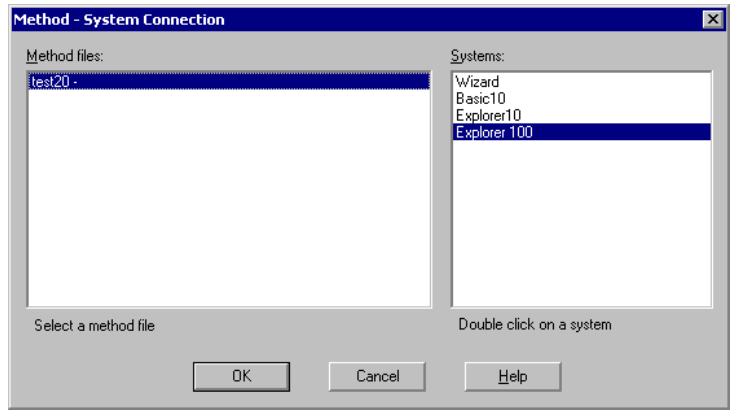


Fig 3-11. Method-System Connection dialogue.

Each copied method listed in the **Method files** field must in turn be connected to the same type of system (same strategy) for which the method was originally created, listed in the **Systems** field. Highlight a method and double click on a system. Click on the **OK** button.

The **Method-System Connection** dialogue is displayed again listing the remaining methods to be connected. Repeat the process until all methods have been connected.

Note: Method syntax errors may arise if a method created on one system is connected to a different type of system using the **Copy from External** facility.

If at any time you press on the **Cancel** button, the **Method - System connection** dialogue is closed. However, it will reappear each time you perform other copy to/from external procedures for method files.

Method files that have been copied in and connected are displayed in the previously designated folder in the **Methods** window.

3.3.7 Deleting files

To delete a file or folder:

1. Select the item(s) to be deleted in the **Methods** or **Results** window of the Main Menu. To select multiple files, hold down the <Ctrl> key while you click on the file names or icons.
2. Highlight the file to be deleted and either select the <Delete> key on the keyboard, choose **File:Delete**, or choose **Delete** from the shortcut menu.
3. Confirm the deletion in the dialogue.

Note 1: Home folders cannot be deleted by this method (see Section 4.2 of the UNICORN 4.0 Administration and Technical manual).

Note 2: Explicit authorisation is required to delete files (see Section 4.2 of the UNICORN 4.0 Administration and Technical manual).

Note 3: A file that has been deleted cannot be recovered except by restoring a back-up copy.

3.3.8 Renaming files

To rename a file or folder:

1. Select a file or folder to be renamed in the **Methods** or **Results** window of the Main Menu.
2. Select **Rename** from the shortcut menu, or choose **File:Rename**. The **Rename** dialogue is displayed
3. Enter the new name for the file and click on **OK**.

3.3.9 Backup security

To protect important data against accidental deletion or loss in the event of hard disk failure, backup copies should be taken at regular intervals.

This can be best achieved by having the UNICORN folders on the server (if available) and working directly from these folders. Alternatively, you can use the **File:Copy to External** function to save files onto the network server. It is standard practice for backups to be made of the server folders. The responsibility for making backup copies rests entirely with the user. Amersham Biosciences cannot undertake to replace method programs lost as a result of computer failure or other incident.

3.4 Printer setup

UNICORN 4.0 uses the default printer and printer settings installed on your computer. To change the choice of printer, either change the default settings in Windows NT/2000 or set up your choice of destination printer for the current working session by selecting **File:Printer setup** in the Main Menu module and selecting the desired printer.

3.4.1 Setting the margins

The default margins for the printers can be changed:

1. Locate the file UNICORN.INI found under C:\UNICORN\BIN, for example by using Windows NT Explorer.

2. Double click on the file to open it and locate the following lines:

```
EVAL     PrintMarginLeft10
EVAL     PrintMarginRight5
Eval     PrintMarginTop5
Eval     PrintMarginBottom5
```

   The values in the lines set the margins based as a percentage of the full width and height of the paper.

3. Change the values as appropriate and save the file.

Caution: Do *not* make any other changes in the UNICORN.INI file (between the lines “BEGIN” and “END”) since this may severely affect the functioning of UNICORN.

3.5 Logging off

To log off from UNICORN click on the **Logon/Logoff** icon or select the **Logoff** menu command.

Processes that are running when you log off will continue to run, and may be left locked with a locking password or unlocked (see Section 6.5 for more details). If the Method Editor module was active at the time of logoff, it will be re-opened when the same user logs on again.

UNICORN will still be open after a user has logged off, and another user may log on. We recommend that you always log off when you leave the computer to prevent other users from accidentally changing or deleting your files or disturbing your runs.

3.6 Quitting UNICORN

To quit UNICORN and close the program, select **File:Quit program** in the Main Menu. You will be prompted to save any unsaved data in the Method Editor or Evaluation module. If a run is proceeding when you quit, do not shut down Windows NT/2000 or turn off the computer while the run is in progress.

Note: You cannot quit the program if you are performing a scouting or MethodQueue run.

4 Creating methods

UNICORN is supplied with a set of **Method Wizards** that can serve as the starting point for creating customised methods. These wizards are defined with variables for critical parameters in the separation, so that customised methods can be created for most purposes simply by setting appropriate values for the method variables. Different wizards are provided for different system strategies. This chapter describes how to create methods - for advanced method editing facilities, see Chapter 5.

Note: Wizards are available for ÄKTAdesign systems delivered with standard strategies.

4.1 Creating a new method

There are three ways to construct a new method:

- Using the **Method Wizard**
- From an existing method **Template**
- Within the **Method Editor**

4.1.1 Method Wizard

The **Method Wizard** is designed as a user-friendly option for constructing new methods. To start the **Method Wizard:**

1. Click on the **Method Wizard** icon (or select **File:Method Wizard**) from within the **Method Editor** screen, or select the **Wizard** radio button in the **Use** field of the **New Method** dialogue. This will prompt the initial Method Wizard dialogue.

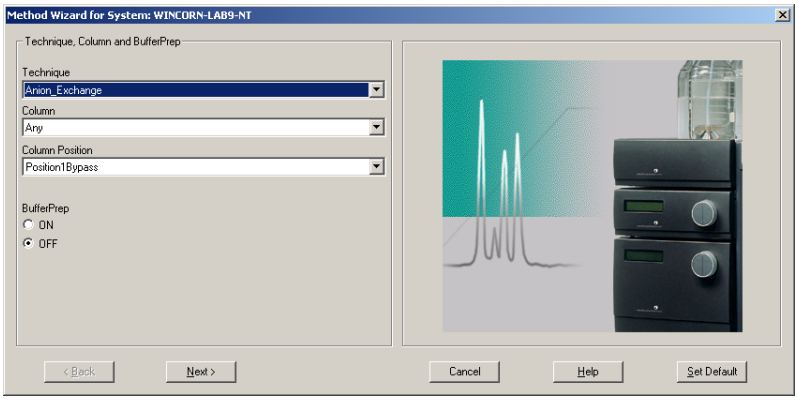


Fig 4-1. The Method Wizard start dialogue.

The options available on the subsequent Method Wizard dialogues depend on which choices you have made in the initial dialogue. The default options on the Method Wizard dialogues can be reset by clicking on the Set Default button on the initial Wizard dialogue.

Note: The instructions available for a given system are determined by the system strategy and components. A method developed for one system may not be valid on another.

2. Make the desired choices on each dialogue, then click on **Next**. For further information on any of the Wizard input fields, click on the **Help** button.

3. On the final dialogue, click on **Finish** to exit the Method Wizard. The new method will be displayed in the Run Set-up editor, where you can, if necessary, make any modifications to the method.

4. Click on the **Save** icon or select **File:Save** to save the new method. Once saved, the method is displayed in the **Methods** window in the Main Menu.

4.1.2 Template-based method creation

To create a new method based on an existing template:

1. In the **New Method** dialogue, select the **Template** radio button in the **Use** field.

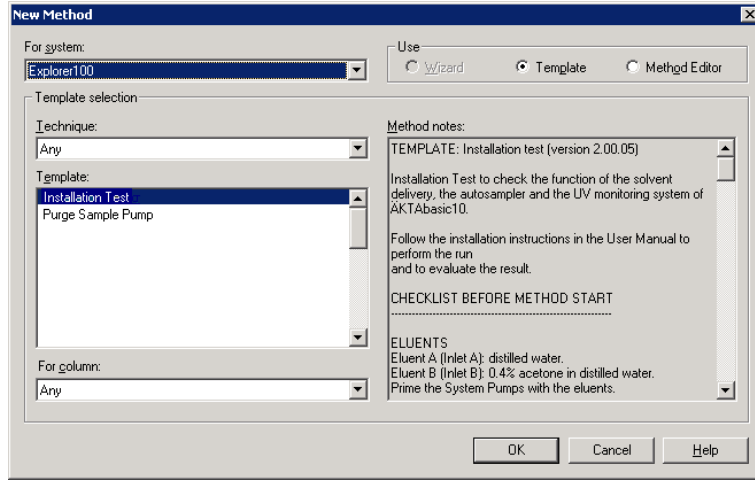


Fig 4-2. New Method dialogue, Template radio button.

2. Choose the system for which the method is intended.

   The instructions available for a given system are determined by the system strategy. A method developed for one system may not be valid on another.

3. Select a chromatographic **Technique**. The templates available for the selected technique will be displayed in the drop-down list box.

   Available techniques may include **Anion_Exchange** chromatography, **Cation_Exchange** chromatography, **RPC** (reverse phase chromatography), **HIC** (hydrophobic interaction chromatography), **Size_Exclusion** chromatography and **Affinity** chromatography. Selection of **Any** causes the templates for all techniques to be displayed.

4. A list of ready-to-run method templates is displayed for the selected technique. Available templates are determined by the system strategy. Select one of these templates to create customised methods either by adjusting variable values (see Chapter 5) or changing method instructions.

   Click on a template to display information about the particular template in the **Method notes** field.

5. In the **For column** drop-down list, choose a specific column to be used. Only columns for the selected technique are displayed. If you do not find your specific column it can be added to the list (see "The Column list" on page 128). The column volume, recommended flow rate, pressure limit and averaging time for the selected column will be automatically copied into the method thus reducing the need to edit the method.

Note 1: You may have to adjust the pressure limit depending on the configuration (tubing etc.) of your system.

Note 2: Except for the column volume, the column-specific values will only be copied into the method if the corresponding instructions are available as variables.

If **Any** is selected, you can use any column but must enter the column volume in the method on the **Variables** tab. It is recommended that a specific column is selected.

6. Click on **OK** once you have made your selections. The method template will now be opened in Run Set-up editor as an untitled method.

4.1.3 Method Editor

To create a new method using the Method Editor:

1) In the **New Method** dialogue, select the Method Editor radio button in the **Use** field.

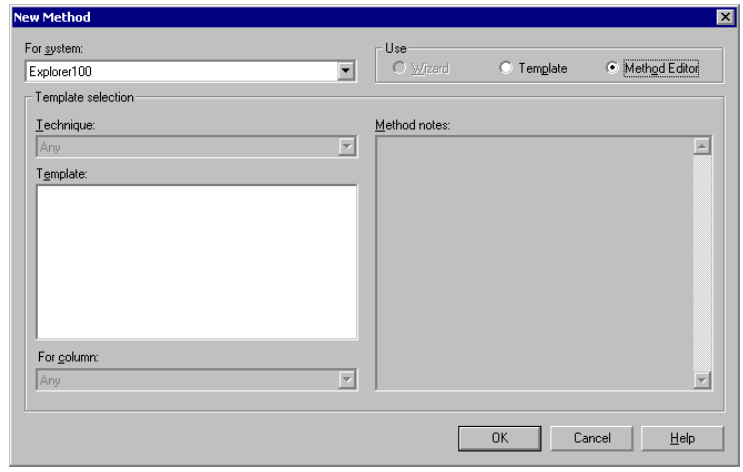


Fig 4-3. New Method dialogue, Method Editor radio button.

2) Click on OK. This will prompt the Text Editor.

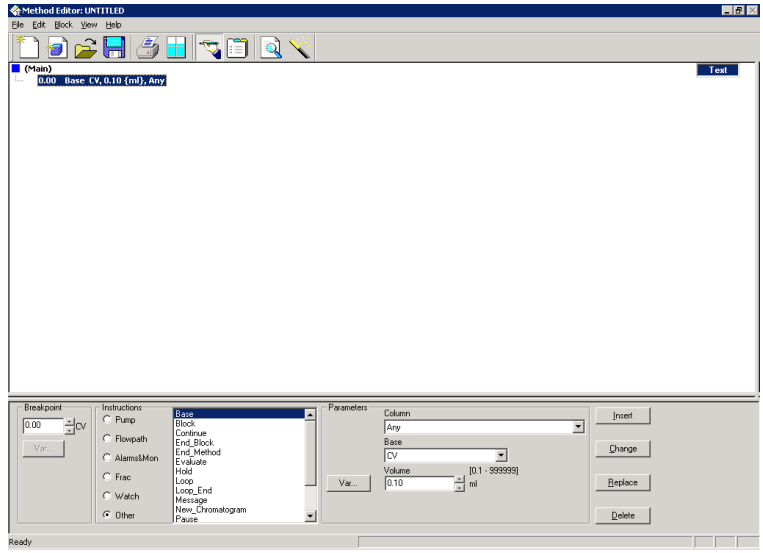


Fig 4-4. Text Editor screen within Method Editor.

The techniques used when creating a new method within the Method Editor are identical to those described in Chapter 5 - refer to that chapter for further details.

4.2 Editing method variables

The method templates are constructed from blocks representing the stages in a typical separation. Each block has a set of method variables, displayed on the **Variables** tab in the Run Set-up. You set default values for the variables in the Method Editor, and can change these values for a particular run in the start protocol before the run is started.

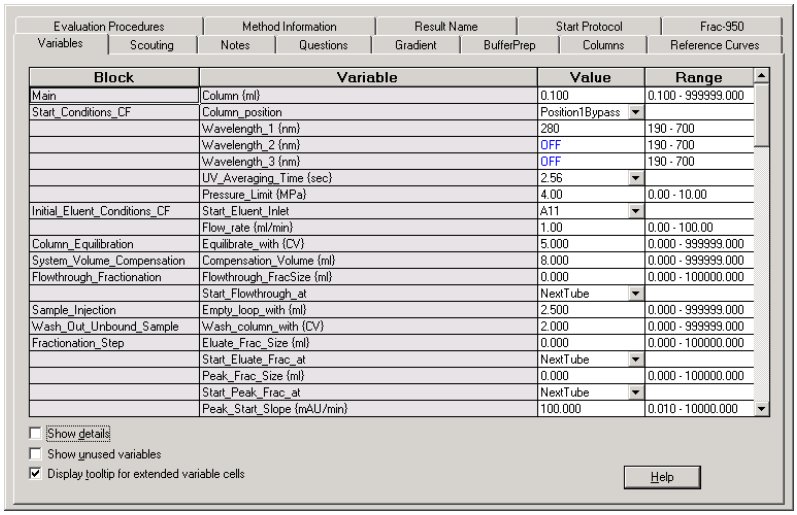


Fig 4-5. Variables tab in Run Set-up.

Work through the variable list, adjusting the **Values** to suit your separation. To change a variable value, simply type the required value in the field. Remember that the values you enter here will be default values, suggested each time the method is run. For some variables, pre-set values are available within drop-down menu boxes.

For variables with values shown in blue, the **Value** input can be toggled between OFF and a variable range by clicking on the **Value** cell with the right mouse button.

Checking the **Show details** checkbox will show variables that were created with the **Visible in details** only option. This option is used for example, to hide less important variables. Similarly, the **Show unused variables** checkbox will display variables that are present in blocks but that are not used in the method.

Note 1: For blocks where the length of the block is defined as a variable (e.g. sample loading, column wash), setting the length to zero will effectively skip the block. Instructions in the block will still be executed, but in an immediate sequence of zero duration.

You can get a graphical view of the gradient profile and the length of the blocks in a method by viewing the **Gradient** window in the Method Editor (**View:Windows:Gradient**) or in the **Gradient** tab of Run Set-up.

Click on the X-axis button in the graphical display to change a base for the graphical display. Changing the display base will not affect the base in the method.

4.3 Method notes

Click on the **Notes** tab in the Run Set-up to show the **Notes** tabs. Read through the **Method Notes** and click on the printer icon or choose **File:Print** to print the method notes.

The method notes provided with each template describe the important information about the template and, if relevant, how the system should be connected for the method to work correctly. If your system does not correspond to the description, either rearrange the valves and tubing connections in accordance with the method notes description or edit the method instructions (see Chapter 5) in accordance with your system setup.

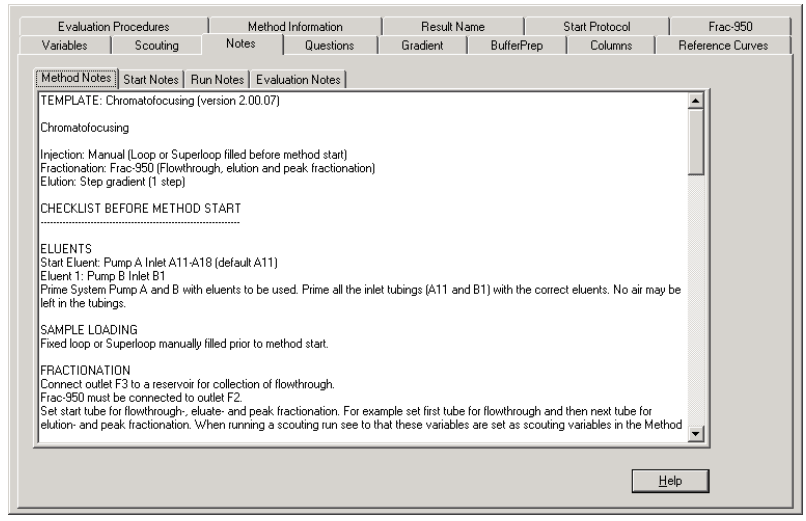

Evaluation Procedures
Method Information
Result Name
Start Protocol
Frac-950
Variables
Scouting
Notes
Questions
Gradient
BufferPrep
Columns
Reference Curves
Method Notes
Start Notes
Run Notes
Evaluation Notes
TEMPLATE: Chromatofocusing (version 2.00.07)
Chromatofocusing
Injection: Manual (Loop or Superloop filled before method start)
Fractionation: Frac-950 (Flowthrough, elution and peak fractionation)
Elution: Step gradient (1 step)
CHECKLIST BEFORE METHOD START
ELUENTS
Start Eluent: Pump A Inlet A11-A18 (default A11)
Eluent 1: Pump B Inlet B1
Prime System Pump A and B with eluents to be used. Prime all the inlet tubings (A11 and B1) with the correct eluents. No air may be left in the tubings.
SAMPLE LOADING
Fixed loop or Superloop manually filled prior to method start.
FRACTIONATION
Connect outlet F3 to a reservoir for collection of flowthrough.
Frac-950 must be connected to outlet F2.
Set start tube for flowthrough-, eluate- and peak fractionation. For example set first tube for flowthrough and then next tube for elution- and peak fractionation. When running a scouting run see to that these variables are set as scouting variables in the Method
Help

Fig 4-6. The Method Notes tab within the Notes tab in Run Set-up.

4.4 Signing the method

To electronically sign the method, choose **File:Sign Method**. The **Sign the Method** dialogue is prompted.

1. The **Sign as user** field shows the current user. In most instances, you will want to use the user shown in the **User** drop-down list box, but it is also possible to select another user from those available on the list.

2. In the **Meaning** field, provide a short text description explaining the meaning behind the signature (e.g., "Method now fully tested and approved").

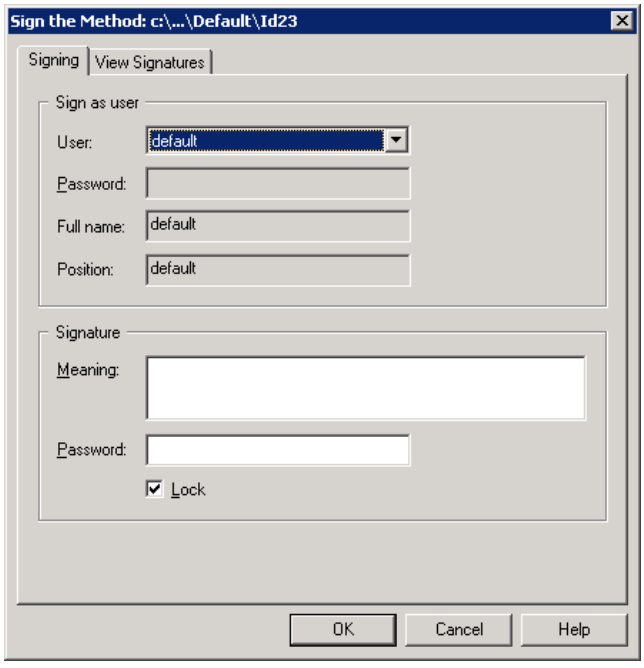


Fig 4-7. Sign the Method dialogue.

3. Type your signature password in the **Password** field, and if desired, check the **Lock** box to permanently lock the method from further changes.

Tip! If the method is locked and you want to make additional modifications, select **File:Save as** and give the method a new name.

4. The **View Signatures** tab provides a list of all signatures associated with the current method.

5. Within either the **Signing** or **View Signatures** tabs, click on **OK** to complete the signature process.

4.5 Saving the method

A new method created from a method template or Wizard is untitled, and must be saved under a method name before it can be run. Click on the **Save Method** toolbar button or choose **File:Save** to save the method.

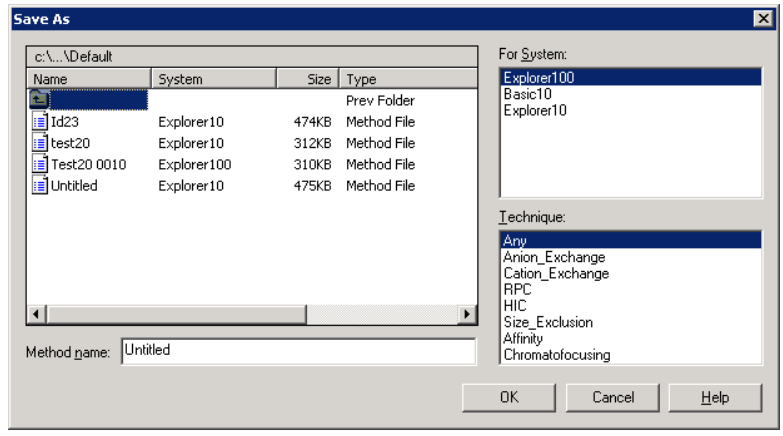


Fig 4-8. Dialogue for saving a method.

1. If required, select another folder than the default home folder in which to save the method.

2. Enter a **Method name** for the method. Method names may be up to 256 characters long. The method name must be unique for the chosen system within the folder (see steps 2 and 3).

3. If you have more than one system connected to the computer, choose the **System** for which the method is intended. The method can be run on any system that uses the same strategy. Remember that different systems may have different configurations and control capabilities.

Note: Each method is written for a specific strategy. The function of the method cannot be guaranteed on systems having other strategies.

4. Choose the **Technique** for which the method was written.

5. Click on **OK**.

Note: If you receive a syntax error message when the method is saved, one or more instructions in the method are invalid. These may be calls to blocks which are not defined, or instructions which are invalid in your strategy (this can also arise if a method is written for one system and saved for another). Invalid instructions are marked in red in text instruction mode in the Method Editor (see "Method instructions" on page 57), and must be deleted or replaced before the method can be run.

The method remains open in the Method Editor when it has been saved, so that you can continue editing if you wish. Once the method has been saved, choosing **File:Save** saves the current state of the method under the same name. If you want to save the method under a new name, choose **File:Save As** and enter the details as described above.

4.6 Starting a run

This section briefly summarises how to start a run with a method. The method must be saved before it can be started. See Chapter 6 for more details of how to run a method.

Note: If you are editing the method in the Method Editor and have made changes that you have not yet saved, these changes will not apply during the run. Similarly, if you edit the method while it is running, the run will not be affected. It is the version of the method that is saved on disk at the time when the method is started that controls the run.

1. Establish a control mode connection to the system where the method is to be run. You cannot start a method without a control mode connection to the appropriate system.

2. Choose **File:Run** from System Control for the required connection and select the method to run. Alternatively, click on the method in the **Methods** window of the Main Menu and select **Run** from the shortcut menu. Do *not* double-click on the method icon in the Main Menu as this will open the Method Editor.

3. Change the method variable values if required. The suggested values are those saved in the method. Any changes you make will apply only for the current run, and will be recorded in the run documentation.

4. Go through the rest of the Start Protocol, entering information where appropriate. Use the **Next** and **Back** buttons to move

through the Start Protocol. Start Protocol tabs for most Wizards and method templates are:

Variables	Adjust variable values as required for the run.
Notes	Read the method notes and enter start notes if required.
Questions	Fill in answers to the questions.
Evaluation Procedures	Select the Evaluation procedure to be run at the end of the method.
Gradient	If desired, click on the X-axis to alter the base unit.
Result Name	Set the result file name and path (folder) as desired. The default result file name includes a 3-digit serial number.
Frac-950	Choose the fractionation order and set the **Last Tube**.

5. The last page of the Start Protocol has a **Start** button. Click on this button to start the run.

4.7 Editing text instructions

Methods for most purposes can be created by adjusting the method variable values as described above. The method is actually programmed as a series of instructions that use these variables as parameters. To see and/or change the instructions, click on the **Text Instructions** icon on the Method Editor toolbar or select **View:Text Instructions** from the menu. To see an overview of the block structure and the gradient in the method, select **View:Windows:Block** and **View:Windows:Gradient** respectively in the Text instruction window (see "Method instructions" on page 57).

With the Text instruction editor, you have complete facilities for designing and editing your own customised methods. You will also use the Text instruction editor for refining and modifying methods based on Wizards and standard templates. For example:

- Changing the method base (column volume, volume or time).
- Changing valve specifications for inlet and outlet (if the method does not suit your system configuration).

- Adding or removing variables.
- Adding conditional events (such as equilibrating a column based on a stable conductivity signal).
- Adding or removing instructions to change the method functionality.
- Adding or removing blocks to change the method structure.

To gain an understanding of how method templates are built up and can be modified, work through Chapter 5 which gives a full description of method editing facilities.

5 Editing methods

This chapter describes the complete facilities for editing methods in UNICORN. For many applications, suitable methods can be created by changing the default variable values in one of the wizard generated methods supplied with UNICORN.

Use the more advanced editing facilities described here for:

- changing selected instructions in the method, e.g. changing the outlet valve position
- adding blocks and instructions, e.g. **Watch** instructions
- changing method instructions to adapt to non-standard system configurations
- creating new methods for applications which are not covered by the templates supplied

5.1 Method Editor interface

5.1.1 Method Editor modes

The Method Editor interface operates in two modes:

- Text instruction editor
- Run Set-up editor

Switch modes using the icons or the commands in the **View** menu. It is possible to show or hide the **Toolbar** and or **Status bar** by clicking **View:Toolbar** or **View:Status bar**.

Fig 5-1. Method Editor toolbar.

Icon	*Menu command*	*Function*
	View:Text instructions	Open the **Text Instructions** editor
	View:Run Set-up	Open the **Run Set-up** editor
	View:Log Format	Open the **Log Format** dialogue.
	File:Method Wizard	Open the **Method Wizard** dialogue.

5.1.2 Text Instructions editor

The text instructions editor is used for entering and editing method instructions. Click on the **Text Instructions** icon or choose **View:Text Instructions** to display the text instructions editor.

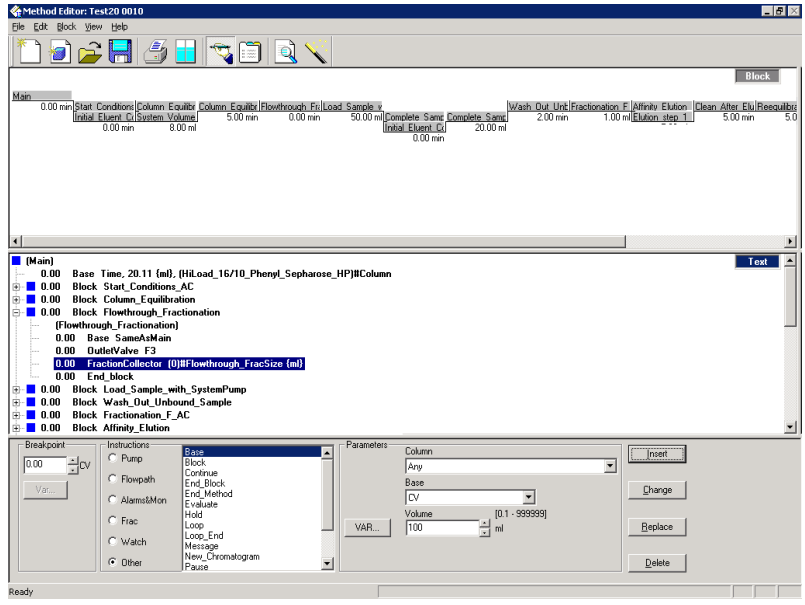


Fig 5-2. The Method Editor in text instruction mode, showing the Block window (top), Text instruction window (centre) and Instruction box (bottom).

Up to four windows can be displayed together with the Instruction box. Click on the **View Windows** icon or select **View:Windows:Customise** to open a dialogue for choosing which windows to display. Individual screens can be selected for viewing through the **View:Windows** drop-down menu options, or hidden by selecting **Hide** from the shortcut menu.

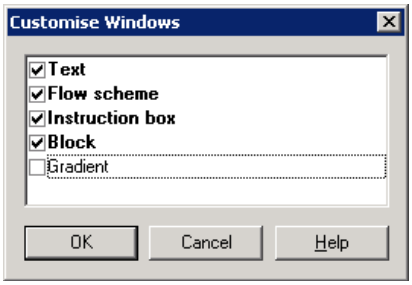


Fig 5-3. The Customise Windows dialogue for selecting which windows to display in the Method Editor.

Method editing operations which can be performed in the various windows are summarised in the table below.

Window	*Functions*	*See Section*
Block window	Select block.	5.2
Text window	Display and hide block instructions. Select current instruction. Move instructions within a breakpoint.	5.3
Gradient window	Display block duration and eluent gradient throughout the method.	5.3.5
Flow scheme window	For information only. The flow scheme picture is static and is therefore not updated according to system status or changes in the method.	5.3.6
Instruction box	Specify breakpoints, instructions, parameters and variables. Insert, change and delete instructions.	5.3.2

5.1.3 Run Set-up

The Run Set-up is a series of tabs for defining the method properties. To access the Run Set-up tabs, press the **Run Set-up** icon or select **View:Run Set-up** from the menu.

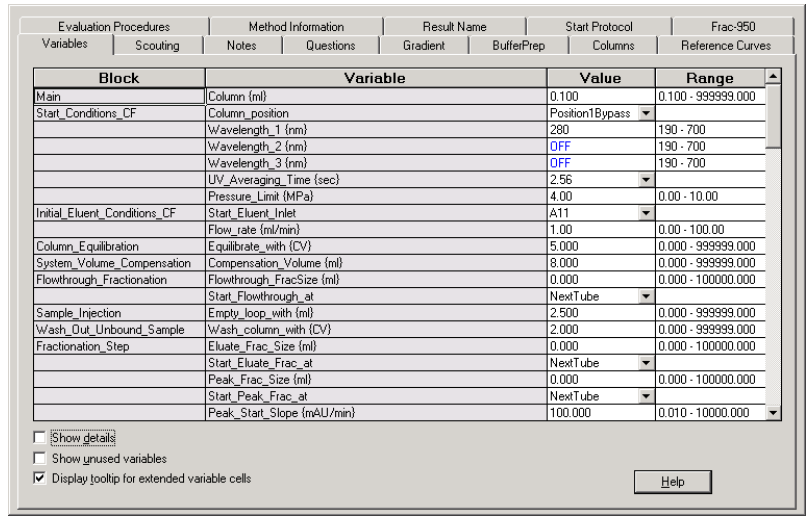


Fig 5-4. Run Set-up with the Variables tab displayed.

To access information in the Run Set-up, click on the relevant tab. Tabs in the Run Set-up editor are described later in this chapter.

5.2 Method blocks

Viewing a method as a long list of individual text instructions may be confusing and inconvenient. Text instructions are therefore conveniently grouped into blocks of instructions that define a specific functional use. For example, one block might contain the instructions necessary for equilibrating a column, and another block contains instructions for loading a sample, etc. By using such blocks it is easier to build up a total method for a run.

5.2.1 Viewing blocks

It is possible for one block to contain one or several sub blocks. This is most evident in the Text instruction window.

In the Text instruction window

In the Text instruction window, the method is shown as a list of

blocks, denoted by the blue square symbols. Beside each block is also a '+' symbol, which you can click on to expand the view of the instructions within the block. Note that a block can also contain sub blocks as denoted by the blue square symbols.

Unexpanded blocks in a method

Expanded block in a method containing text instructions and a sub block.

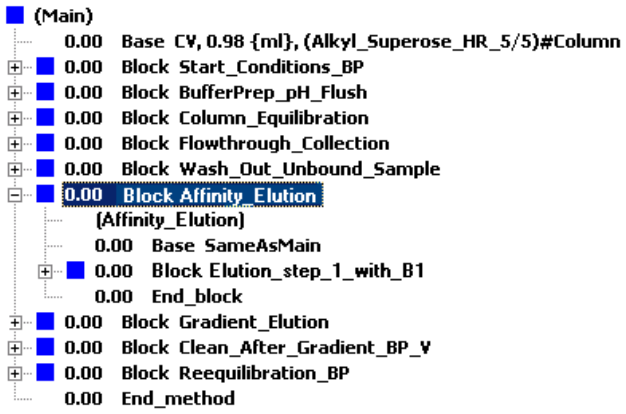


Fig 5-5. Text instructions showing blocks in a method.

To collapse the view, click on the '-' symbol for that block.

Alternatively you can double click on the block name to view or hide the instructions (see Section 5.3.1).

In the block window

The organisation of blocks in the method is shown graphically in the block window in text instruction mode.

Each block is represented by a grey bar with the block name and the length of the block. The line is shifted down to indicate calls to other blocks. In the following example, the blocks are called in sequence from the Main block at breakpoint 0. Blocks to which there is no valid call are not shown in this window.

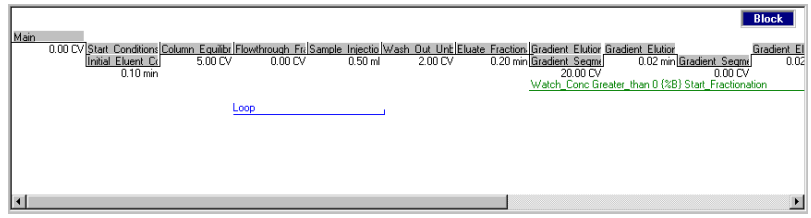


Fig 5-6. The Method Editor block window.

Conditional (**Watch**) instructions are indicated by a green line showing the start and duration of the watch. The example above has a **Watch** instruction to start the fraction collector which is active throughout the gradient elution block. **Loop** (to repeat a group of instructions) and **Hold_until** instructions are also indicated in the block window.

If you click on the line representing a block in the block window, the first instruction in the block will be highlighted in the text window.

In the gradient window

Blocks are represented in the gradient window by marks on the X-axis showing the length of each block. The name of the block in which the cursor line is currently placed is shown at the top of the window (see "Using the gradient window" on page 64).

5.2.2 Calling blocks

To execute the instructions contained within a block in a method, the block must be *called* by the program. When a block is called the instructions in the block are executed in the order that they are written until the block is finished or the **End_Block** instruction is executed. Any settings made in a block are valid throughout the method until the settings are changed.

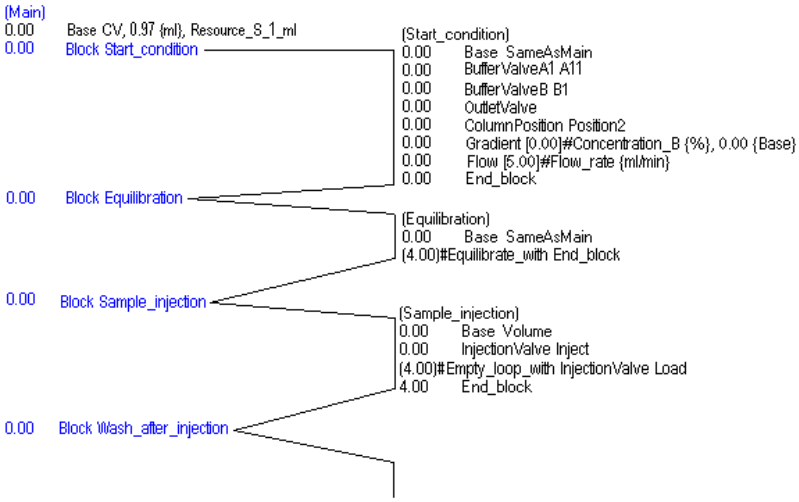


Fig 5-7. Illustration of the flow of process control through method blocks.

Calls may be of two types:

- *Unconditional calls* are made with the **Block** instruction.
- *Conditional calls* are made with a **Watch** instruction, which makes it possible to call a specified block or an instruction when a particular monitor signal meets a given condition. As long as the condition is not met, the block is not activated. **Watch** instructions can use various conditions to respond to absolute signal values or to rate of signal change.

  Note that the breakpoint when the **Watch** instruction is issued determines when the watch begins, not when the block is activated. The block will in fact never be activated if the watch condition is not met during the run.

  Once set, a watch remains active until the condition is met or a new **Watch** instruction is issued for the same monitor. The watch is cancelled automatically when the condition is met. A watch can also be turned off with the **Watch_off** instruction.

  See Section 5.11.2 for more details of **Watch** instructions.

5.2.3 Adding blocks

To add a **Block**, select the **Block** instruction in the Instruction box, enter a name for the block in the parameter field and click on **Insert**. The block will be inserted after the instruction/block that was highlighted in the **Text Instruction** window. You can also select an existing block from the parameter field drop-down list.

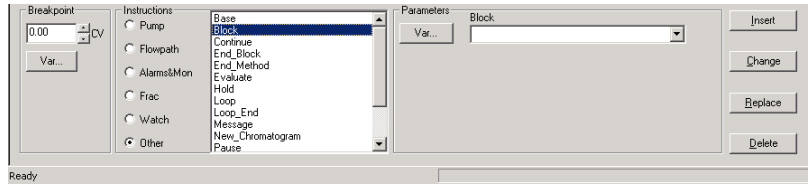


Fig 5-8. Instruction box with the Block instruction selected.

Alternatively, to add a new block use the **Text Instruction** editor and click on the **New block** icon or select **Block:New**.

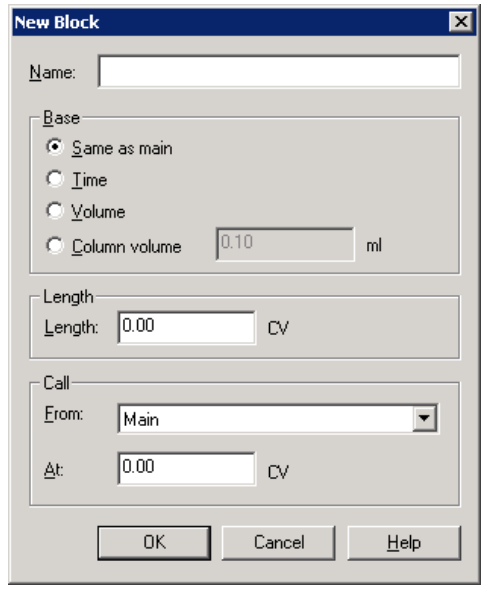


Fig 5-9. New block dialogue.

Block name

Enter a name for the block in the **Name** field. Block names may be up to 30 characters long, and may contain letters (A-Z), digits (0-9) and underscore characters.

Block names must be unique within the method. The case of letters is retained but is not significant (the names **Start_Frac** and **START_FRAC** are treated as identical).

Base

Choose a base for the block. If you choose **SameAsMain**, the new block will inherit the base from the **Main** block in the method. The corresponding **Base** instruction will be inserted in the block at breakpoint 0. If you choose **CV**, enter a value for the column volume. If you chose a specific column, a column volume is entered automatically.

Length

You can enter a length for the block if required. An **End_Block** instruction will automatically be inserted in the block at the corresponding breakpoint. This field may not be left blank.

Call

You can call the new block from an existing block (e.g. the Main block). Choose the block from which the newly created block should be called in the **From** field and enter the breakpoint at which the call is to be made in the **At** field. If you do not want to call the block (e.g. when the block being created is to be activated by a **Watch** instruction), choose the **<Unused>** line in the **From** drop-down list. These blocks are placed last in the method in the Unused category.

Note 1: If the **Block** instruction is placed at the same breakpoint as the **End_Block** instruction, the **Block** instruction will be placed immediately before **End_Block**.

Note 2: Do not call a block from within itself. You will generate a potentially infinite loop, which exceeds the maximum number of calls allowed in a method. A loop symbol is displayed at the beginning of the line if this occurs.

Press **OK** to add the new block.

5.2.4 Deleting blocks

You can delete a block instruction by either (i) clicking the **Delete** button in the **Instruction** box, (ii) by using the shortcut menu, (iii) using the **Block:Delete Block** command, or, (iv) clicking on the <Delete> key on the keyboard.

Deleting blocks using the shortcut menu

1. Click on the desired block in the method with the right mouse button to display the shortcut menu.
2. Select **Delete**. A warning dialogue is displayed requesting if you want to totally delete the block instruction from the method. If you choose Yes and the block contains sub-blocks, another warning dialogue is prompted, asking if you want to delete the sub-blocks as well.

Answer as appropriate:

Yes The block is totally removed from the method. If the block is called several times in the method, all the blocks will be deleted. Blocks deleted in this fashion can not be called again in the method.

No The block is deleted from the method and transferred to the Unused line. If the block is called several times in the method, however, only the row with the block currently marked in the **Text** instruction window will be deleted. In this case, the block will not be placed in the Unused section (since the block is still used in the method). Blocks deleted in this fashion can be called again in the method.

Deleting blocks using the Block:Delete Block command

To delete a block from the method using the **Block:Delete** command:

1. Select the menu command **Block:Delete Block**. The **Delete Block** dialogue is displayed with all blocks listed in alphabetical order.

2. Select the block to be deleted and click on **Delete**.

5.2.5 Renaming blocks

To rename blocks, choose **Block:Rename Block** or select a block in the method using the shortcut menu and select **Rename** from the shortcut menu. In both instances the **Rename Block** dialogue is displayed.

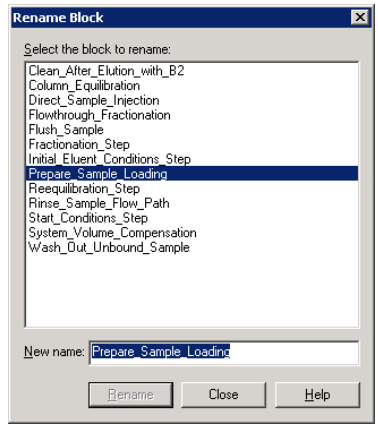


Fig 5-10. The Rename Block dialogue

By default, the block that is currently highlighted in the method text instructions is automatically selected in the dialogue. Enter the new name in the **New Name** field and press **Rename**. The dialogue remains open until you press **Close**, so that you can rename more than one block without closing the dialogue.

If the block you renamed is used in a **Block** or **Watch** instruction, the block name in these instructions will be changed automatically.

5.2.6 Copying, moving and importing blocks

By using the shortcut menu or **Edit** options in the Method Editor, you can find, move, copy, cut and paste blocks within a method or import blocks from another method.

Finding text in the text window

This function searches for specific text strings in the method text.

1. Choose **Edit:Find**, or use the shortcut menu and select **Find**.

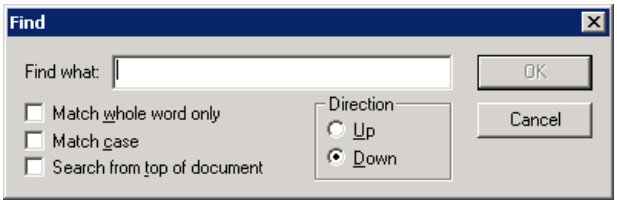


Fig 5-11. Find dialogue within the Text window.

2. Enter the text that you want to search for, the search direction, and any case matching criteria.

3. Click on **OK**. You can also search for the next occurrence of your selected text string by choosing **Find Next** (<F3>) from the shortcut menu. Similarly, choose **Find Previous** (<Shift+F3>) to move back to the previous occurrence of the current text string.

Moving blocks

To move a block:

1. Using the shortcut menu, select the block to be moved and then select **Cut** from the shortcut menu. Alternatively, select **Edit:Cut** or <Ctrl+X>.

2. Select the instruction line just *above* the insertion point where you want the cut block to be pasted.

3. Select **Paste** from the shortcut menu, **Edit:Paste**, or <Ctrl+V>.

   The block is now removed from its original breakpoint and pasted at the new breakpoint. The pasted block is inserted with the same breakpoint value as the block or instruction selected for point of insertion.

Copying blocks

To copy blocks within a method:

1. Select the block to be copied using the shortcut menu. Select **Copy** from the shortcut menu, **Edit:Copy**, or <Ctrl+C>.

2. Select the instruction line just *above* the insertion point where you want the cut block to be pasted.

3. Select **Paste** from the shortcut menu, **Edit:Paste**, or <Ctrl+V>. A dialogue requests if you wish to rename the pasted block. Click on **Yes** to rename the block before insertion or **No** to directly insert the copied block.

   The pasted block is inserted with the same breakpoint value as the block selected for point of insertion.

Importing blocks

Blocks may be imported from other method files to which you have access including the current method file in which you are working.

1. Select **Block:Import Block As**.

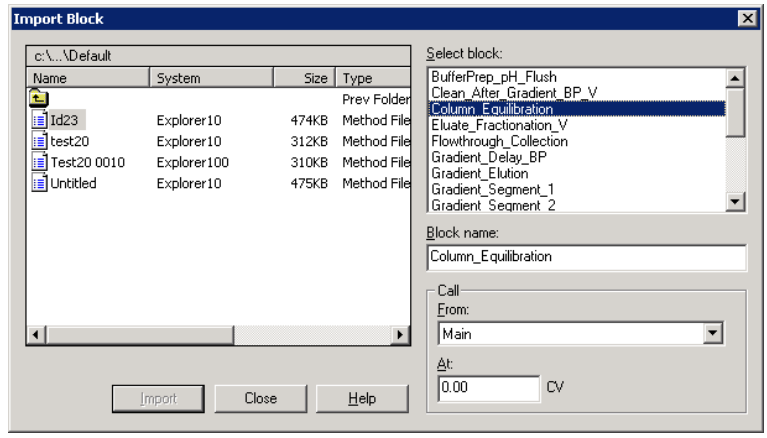


Fig 5-12. The Import Block dialogue.

2. Choose the method from which you wish to import and select the block to import. The name of the selected block is suggested in the **Block name** field.

3. Select a block from the **Call** drop-down list into which the imported block will be placed and enter a breakpoint value.

4. Click on **Import** to import the block. The dialogue remains open until you click on **Close**, so that you can import more than one block without closing the dialogue.

Note: If you use the **Import** function to copy blocks within a method, the blocks are copied from the saved version of the method on disk. Any changes you have made in the method but not yet saved will not be copied.

When importing a block that contains one or more sub-blocks you will be asked if the sub-blocks are to be imported as well.

The imported block may not have the same name as an existing block in the method. If the default name is not allowed for this reason, the **Import** button will be grey and locked. Change the name of the imported block so that the **Import** button becomes available.

The block is imported exactly as it appears in the source method. If the base of the imported block is defined as **SameAsMain**, the block will inherit the main base in the new method, regardless of the base in the source method. Also, the imported block is inserted with the same breakpoint value as the block selected for point of insertion.

5.3 Method instructions

Use the Instruction box in text instruction mode to enter, edit and delete instructions.

5.3.1 Viewing instructions

Instructions are displayed in the text Instruction box.

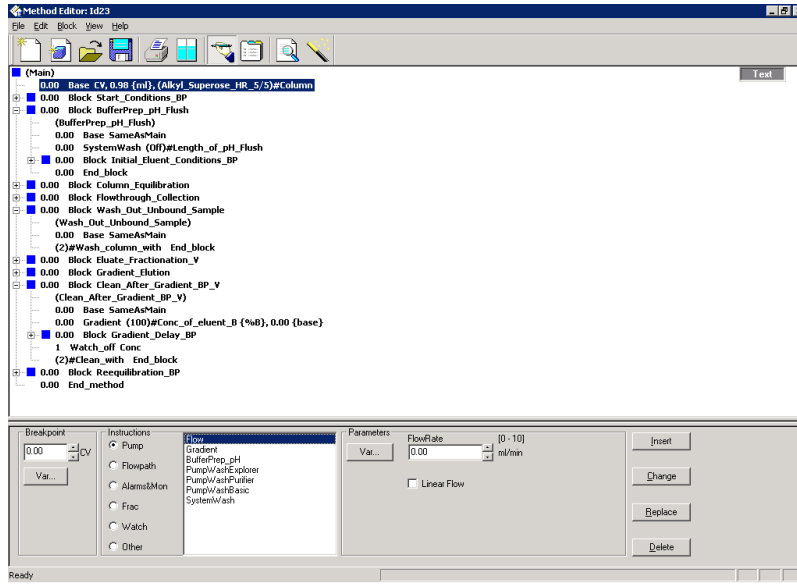


Fig 5-13. The text instruction window (top) with the Instruction box (bottom).

Instructions are displayed in the text instruction window as follows:

Blue square beside text	Valid call instructions (i.e. **Block** and **Watch** instructions to other blocks in the method).
Bold text	Valid instructions.
Red check mark	Instructions with invalid syntax. These may be: (a) calls to blocks which are not defined in the method, or (b) instructions which apply to a different system strategy (these can arise if a method is written for one system and saved for another), or (c) instructions for components that have not been selected in the System Set-up. All such instructions must be deleted or changed before a method can be run (see "Changing instructions" on page 61).

Normal text	Instructions which will not be executed because they are either after the end of a block or method or constitute a block to which there is no call.
Text with a loop symbol	When a block is called from within itself this will generate a potentially infinite loop, which might exceed the maximum number of calls allowed in a method.

Double-click on a **Block** or **Watch** instruction to display or hide the instructions in the called block or click on the '+'/'-' symbol for the block respectively. Double-clicking on the **Main** keyword at the beginning of the method will show or hide instructions in all blocks in the method.

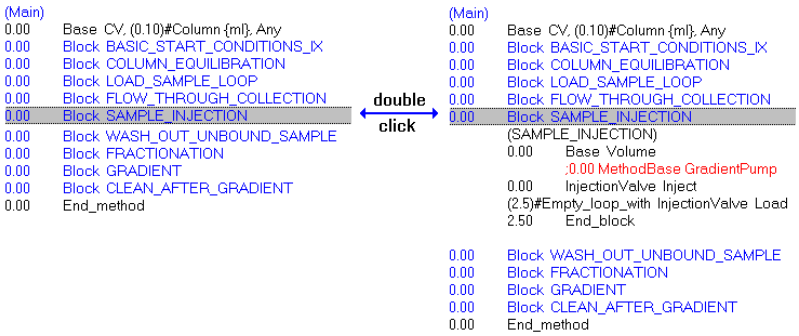


Fig 5-14. Displaying and hiding block instructions.

5.3.2 Adding instructions

To add a new instruction:

1. In the text instruction window, select an appropriate block and display the instructions within the block.
2. Select an instruction line in the block.

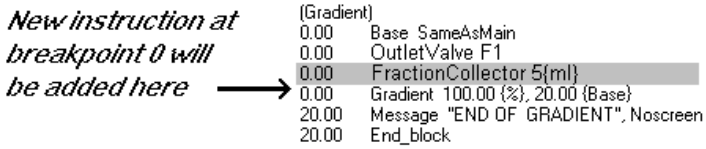

New instruction at breakpoint 0 will be added here
(Gradient)
0.00 Base SameAsMain
0.00 OutletValve F1
0.00 FractionCollector 5{ml}
0.00 Gradient 100.00 {%}, 20.00 {Base}
20.00 Message "END OF GRADIENT", Noscreen
20.00 End_block

Fig 5-15. Instructions added at the same breakpoint as existing instructions are inserted after the highlight.

Note: Make sure that the selected instruction line is within the block, not the call to the block.

3. New instructions are added from the Instruction box. Open the Instruction box (**View:Windows**) if it is not already displayed. For the new instruction:

   (a) Set the desired breakpoint in the **Breakpoint** field.

   (b) Choose the instruction type from the various options, depending on the strategy, and select the desired instruction from the displayed list. For short help on the purpose of each instruction, click on the instruction and press <F1>.

   (c) Enter values for instruction parameters in the **Parameters** field. If a scroll bar appears on the right-hand side of the **Parameters** field, additional parameters are required.

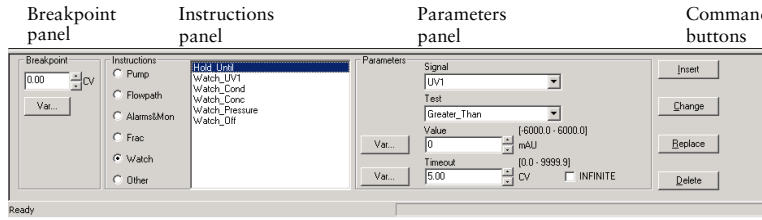


Fig 5-16. The Method Editor Instruction box.

4. Click on **Insert**. The new instruction will be inserted in the block either

   (a) at the position of the breakpoint of the new instruction if there are no other instructions at that breakpoint

   (b) immediately after the currently highlighted instruction if the highlight is at the same breakpoint as the new instruction

   (c) as the last instruction at the breakpoint if there are several instructions at the same breakpoint as the new instruction and none of these is highlighted.

Note 1: Instructions that are placed at the same breakpoint are executed simultaneously, with the exception of **Block** instructions which are executed in the sequence in which they are written.

Note 2: If you are using ÄKTA systems, the **Pause**, **Hold**, and **Hold_until** instructions will stop execution at this breakpoint, i.e. instructions following after **Pause**, **Hold** and **Hold_until** at the same breakpoint will not be executed until a **Continue** instruction is issued.

5.3.3 Deleting instructions

To remove an instruction:

1. Select the instruction in the text instruction window.

2. Use one of the following three alternatives:

   - select **Delete** from the shortcut menu, or
   - press **Delete** in the Instruction box, or
   - press on the Delete key on the keyboard.

An instruction that has been deleted can only be recovered by re-inserting the instruction. If you want to suspend execution of an instruction temporarily (e.g. during development work), you can replace the breakpoint with a value after the **End_block** or **End_method** instruction.

Note: You cannot delete the **Base** instruction at the beginning of a block.

Caution: If you delete the **End_block** instruction, the block will end at the last instruction in the block. If a gradient is currently being formed, the gradient will continue into the next block.

5.3.4 Changing instructions

There are three possibilities for changing an instruction:

- change the breakpoint
- change parameters (including variables, see “Method variables” on page 66)
- select another instruction

To change an instruction:

1. Select the instruction in the text instruction window. The instruction with its current parameters will appear in the Instruction box.

2. In the Instruction box, make the required changes to the breakpoint or parameters or choose a new instruction.

3. Press **Change** or **Replace**. These buttons are equivalent unless changes are made to the breakpoint or the length of a gradient.

Changing breakpoints

Change and **Replace** have different functions if the breakpoint is changed:

- **Change** shifts all subsequent instructions in the block according to the change in the breakpoint. **Change** does not affect the relative order of instructions in the method. You cannot change the breakpoint of an instruction to earlier than the nearest previous breakpoint in block. For example, changing **FractionCollector** from breakpoint 0 to 5 with **Change**:

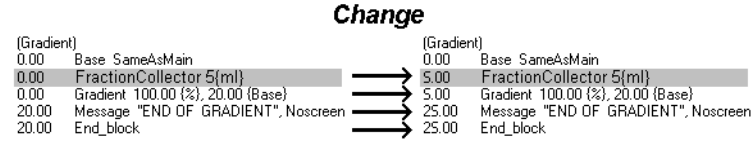


Fig 5-17. Change moves the selected instruction and all subsequent instructions.

- **Replace** moves the selected instruction but does not change the breakpoint of any other instruction. **Replace** can change the relative order of instructions in the method. For example, changing **FractionCollector** from breakpoint 0 to 5 with **Replace**:

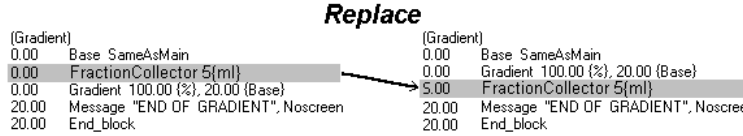


Fig 5-18. Replace moves only the selected instruction.

Changing gradient lengths

The length of a gradient is changed by altering the **Length** parameter

in the **Gradient** instruction. If **Change** is used to alter the length of a gradient, the breakpoints for any instructions issued during the course of the gradient will be adjusted proportionately, so that they are always placed at the same relative position within the gradient. Instructions issued after the end of the gradient will be shifted by the amount of the change. Since the gradient functions over time, any instruction that you want to insert after a gradient should be placed after the combined breakpoint and gradient length.

Note: Moving the **End_block** instruction in a gradient block with **Change** does not affect the length of the gradient.

If **Replace** is used to alter the length of a gradient, other instructions are not affected.

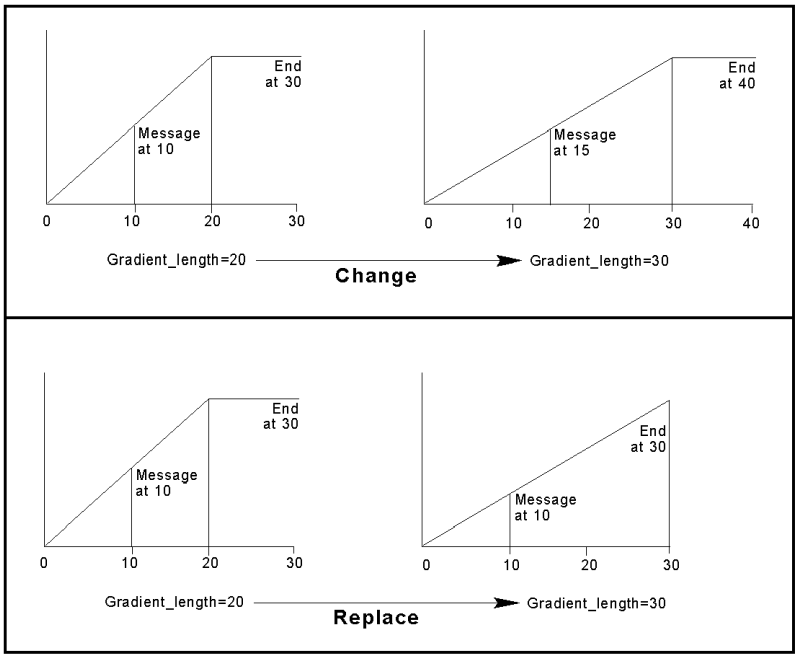


Fig 5-19. Illustration of the different effects of Change and Replace on instructions within and after gradients.

Moving instructions within a breakpoint

To change the order of instructions within the same breakpoint in a block, mark the instruction to move with the left mouse button and drag the instruction to its new location, holding the left mouse button down. You can only move instructions in this way within a group of instructions at the same breakpoint.

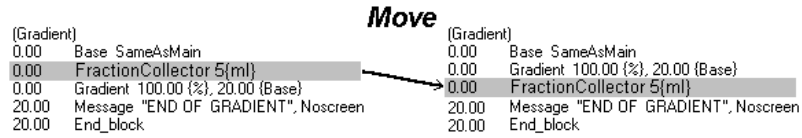


Fig 5-20. Instructions can be moved within the same breakpoint by dragging with the shortcut menu.

Moving instructions between breakpoints

To move an instruction to another breakpoint:

1. Using the shortcut menu, select the instruction to be moved. Select **Cut** from the shortcut menu, **Edit:Cut**, or <Ctrl+X>.

2. Select the instruction line just *above* the insertion point where you want the cut instruction to be pasted.

3. Select **Paste** from the shortcut menu, **Edit:Paste**, or <Ctrl+V>.

   The instruction is now removed from its original breakpoint and pasted at the new breakpoint. The pasted instruction is inserted with the same breakpoint value as the instruction selected for point of insertion.

5.3.5 Using the gradient window

The gradient window provides a graphical overview of the block structure and eluent gradient in the current method:

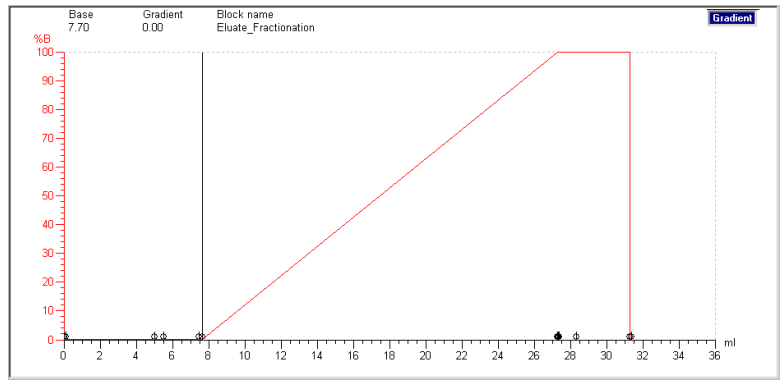


Fig 5-21. The Method Editor gradient window.

Vertical cursor line

A vertical cursor line can be dragged from the Y-axis with the mouse. As you drag the cursor line:

- The current position is identified at the top of the window, in terms of the block name, x-position in the currently displayed base and eluent concentration in % eluent B.
- If the text instruction window is displayed, the instructions are scrolled so that the instruction at the cursor line is at the top of the window.
- If the Instruction box is displayed, the instruction at the cursor line is shown in the dialogue.

The zoom function

To zoom in on a selected region of the Gradient window:

1. Press down and hold the left mouse button and drag a rectangle out on the screen to encompass the area to be viewed.
2. Release the mouse button. The display is now zoomed in on the selected area.
3. Repeat the process for further magnification of selected areas.

You can reduce the scale of the zoom in function in two ways, either:

- reverse each zoom in action a step at a time by selecting **Undo Zoom** from the shortcut menu, or,
- reverse all of the zoom in actions to the default scale setting by selecting **Reset Zoom** from the shortcut menu.

Changing the base

You can select the base shown on the X-axis, either time, volume or column volumes. Make the appropriate selection from the shortcut menu option **Base type**. Changing the base for the display does not affect the base in the method instructions. You can therefore check how long a method will take simply by setting the axis scale to time, even if the method blocks are written in volume or column volume base.

Viewing Hatch marks

You can display a hatched screen in the Gradient window by selecting **Hatch** from the shortcut menu.

5.3.6 The flow scheme window

The flow scheme window displays the configuration of system components. This window is static and for information only, useful for example in identifying valves for flow path instructions.

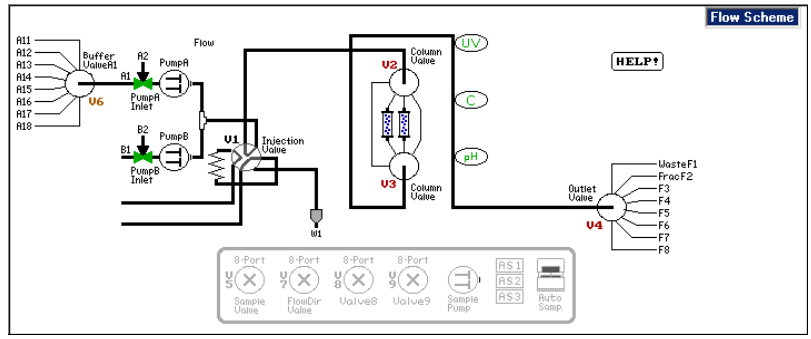


Fig 5-22. The Method Editor flow scheme window.

5.4 Method variables

Variables can be assigned to most instruction parameters including breakpoints. Variable values can be changed immediately before the start of a run without using the Method Editor, allowing one method to be used for runs under a variety of conditions (see Chapter 4). Variables also form the foundation for automatic method scouting (see Chapter 7).

Variables are defined with names which can be explicit descriptions of the variable function, e.g. **Sample_volume**, **Gradient_length**. Suitable choice of variable names can make the method easier to read and understand, and also help the operator in setting variable values at the start of a run.

Each parameter defined as a variable is also assigned a default value, which is used if no changes are made to variable values at the start of a run. Up to 500 variables can be defined in a single method.

5.4.1 Identifying variables

Parameters defined as variables are identified in two ways:

- in the text instruction window, the parameter is given as the default value in parentheses followed by the variable name, e.g. (4.00)#`Empty_loop_with`.

```
(Sample_injection)
0.00      Base Volume
0.00      InjectionValve Inject
0.00      OutletValve F2
{4.00}#Empty_loop_with InjectionValve Load
4.00      End_block
```

Fig 5-23. Default values for variables appear in parentheses in Text instructions

- when the instruction is shown in the Instruction box, the **VAR** button beside the parameter field is active in capital letters, i.e. **VAR** not **Var**.

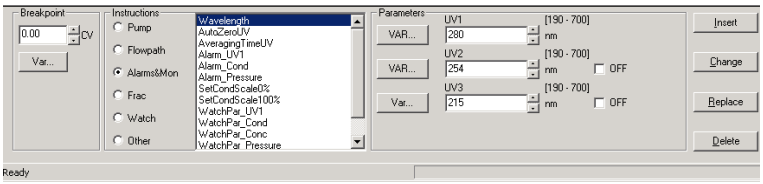


Fig 5-24. Parameters with variable definition are identified by an active **VAR** button. In this example UV1 and UV2 are defined as variables and the UV3 position is fixed.

All variables are also listed on the **Variables** tab of the Run Set-up (see "Variables" on page 70), grouped according to the block in which they appear.

5.4.2 Defining variables

To define a new variable (i.e. convert an existing fixed value to a variable):

1. In the Text instructions window, select the instruction where you want to define the variable. The parameters for the instruction are shown in the Instruction box.

2. Locate the breakpoint or the required parameter in the Instruction box. Click on the **Var** button.

3. Enter a name for the variable in the dialogue and click on **OK**.

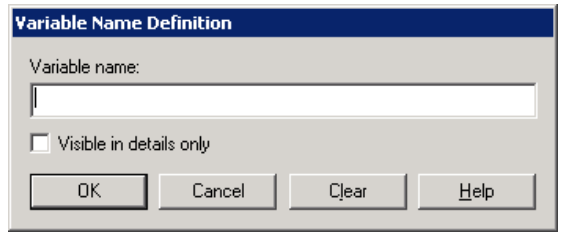


Fig 5-25. The Variable Name Definition dialogue.

Variable names may be up to 20 characters long, and may contain letters (A-Z), digits (0-9) and the underscore character. Use underscore characters instead of spaces if you want to separate words in a name (e.g. Flow_rate). Names must be unique within the method.

The case of letters is retained but is not significant. The names Flow_Rate and FLOW_RATE are treated as identical.

When you define a variable, the value in the parameter field applies as the default value for the variable.

Note: Check the **Visible in details only** checkbox to set the variable as a "details" variable. Detail variables become visible on the **Variables** tab if the **Show details** checkbox is checked. This option is useful for hiding less important variables.

Note: Only one variable which affects block length (breakpoint or gradient length) may be defined within each block. Any number of other parameters may however be defined as variables within a block.

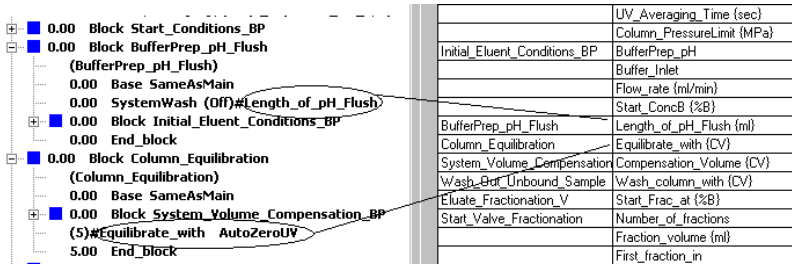


Fig 5-26. Relationship between variables in text instructions and in the Variables tab of Run Set-up.

Default variable values can be changed either by editing the instruction in the Instruction box or by changing the value in the **Variables** tab of Run Set-up. Changes made in the text instruction are automatically updated in the **Variables** tab and vice versa (see figure above).

Note1: If a breakpoint or gradient length is defined as a variable, changing the variable value in the Start protocol when the method run is started will shift other instruction breakpoints accordingly. This functionality is equivalent to using **Change** to alter a breakpoint or gradient length (see page 63 for how **Change** affects instructions within gradients).

Note2: If the same block is called several times in a method, the variables in that block will only be displayed once in the **Variables** tab of Run Set-up.

5.4.3 Removing a variable

To convert a variable to a fixed value:

1. In the Text instruction window, select the instruction where you want to remove the variable. The parameters for the instruction will be shown in the Instruction box.

2. Locate the required parameter in the Instruction box. Press the **VAR** button.

3. Click on **Clear** to delete the variable name and click on **OK**.

Note: Do not remove column variables (flow, pressure, UV averaging time) if you want these parameters to be updated if you change column.

5.4.4 Renaming a variable

To change the name of an existing variable:

1. In the Text instruction window, select the instruction where you want to rename the variable. The parameters for the instruction will be shown in the Instruction box.

2. Locate the required parameter in the Instruction box. Press the **VAR** button.

3. Enter a new variable name in the dialogue and click on **OK**.

5.5 Run Set-up

The Run Set-up is a tabbed window for defining the method properties. To access the Run Set-up tabs, press the **Run Set-up** icon at the top of the Method Editor toolbar. To access a given tab in the Run Set-up, click on the respective tab.

5.5.1 Variables

The **Variables** tab lists all variables used in the method with their default values, organised by method block. You can change the default values to create a variant of the method (see Chapter 4). Note that in a scouting method, values for variables included on the **Scouting** tab cannot be changed on the **Variables** tab.

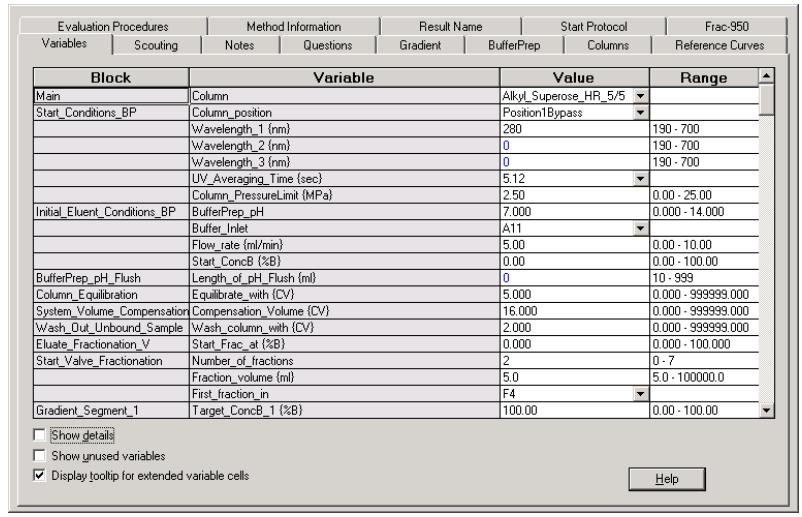


Fig 5-27. The Variables tab in Run Set-up.

To change the default values, simply enter new values in the appropriate fields. Use the scroll bar to display additional variables if the variables occupy more than one screen.

The changed values will be displayed for the corresponding instructions in the Text instruction window. Remember to save the method if you want to use the new values.

Note: The **Variables** box must be checked in the **Start Protocol** tab if you want to be able to change variable values at the start of a method.

Note: Detail variables are only shown if the **Show details** box is checked.

5.5.2 Scouting

A scouting scheme is a series of runs where chosen variable values are varied. When a method is run using scouting, the method is repeated automatically for each selected run in the scouting scheme. Typically, scouting will vary one or more variables in a series of runs, e.g. flow rate or elution gradient. See Chapter 7 for a full description of scouting facilities.

All scouting parameters can be set when a run is started, provided that **Scouting** is checked in the **Start Protocol** tab. The parameter values entered in the Method Editor provide the default settings for the scouting method.

To prepare a method for scouting:

1. Select a method with appropriate variables in the method instructions.
2. Open the Scouting tab in Run Set-up.
3. The **Scouting Variables** dialogue is displayed automatically if there are no scouting variables defined. If scouting variables are already defined and you want to change the selection of scouting variables, click on the **Define** button to display the **Scouting Variables** dialogue. Checking the **Show details** check box will display variables created with the **Visible in details only** option. Checking the **Show unused variables** checkbox will display variables that are present in blocks that are not used in the method (These blocks can, however, be utilised in the manual instructions box.).

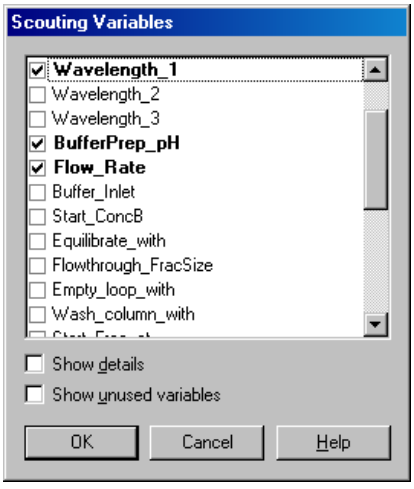


Fig 5-28. The dialogue for defining scouting variables.

Check the required variables in the variable list and click on **OK**.

4. Make any changes you require in the variable values. For variables with text values (e.g. column position), select the required value from the drop-down list.

Note: Values for variables selected for scouting under **Define** cannot be changed on the **Variables** tab. Changes made on the **Scouting** tab do not affect the default values in the method instructions.

5. To add a new Run column, click on **Add** to copy the values from the last run column, and then change variable values as required.

6. Repeat this procedure until you have defined all the runs you require. The example below shows scouting set for four flow rates.

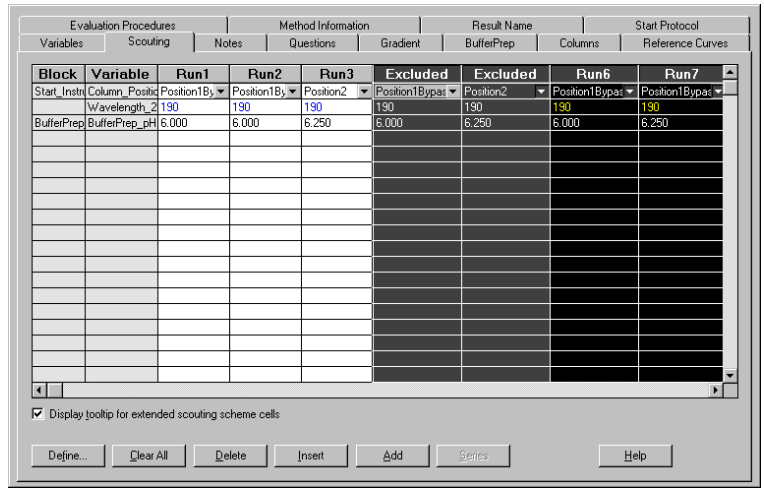


Fig 5-29. Scouting scheme with varying BufferPrep_pH and Column Position in this example.

You can define up to 99 runs in a scouting scheme. Each run can be set to **Run** or **Excluded** by clicking on the button at the top of a run column and clicking with the shortcut menu. Values can also be changed during the run in System Control. The Excluded option simply means that the run is skipped and the next run is taken in sequence.

The **Series** button is useful when you want to set up a series of runs with differing integer inputs. Select a cell with an integer value and click on the **Series** button. In the resulting **Insert Series** dialogue, type in the selected series of integer values (within the specified range

limits), separated by commas. Click on **OK**. A new set of runs is inserted into the **Scouting** tab with the values provided.

Any changes that you make to variable values when a scouting scheme is run are saved in the result file. Results from a scouting run are saved in a scouting folder (see Chapter 7).

Column scouting

It is possible to define different columns for use in the various scouting runs. However, in selecting a different column, other variables may also be changed between runs.

1. Choose a method with a column (not "**Any**"). Alternatively you can have a method with CV as the main base and a column (not "**Any**") selected as a variable called "column".

2. To define columns for a scouting run click on the **Define** button to display the **Scouting Variables** dialogue. Select **Column** and then **OK**.

3. Click on the Column drop-down menu within the desired run. A menu is displayed in which you can select the appropriate column.

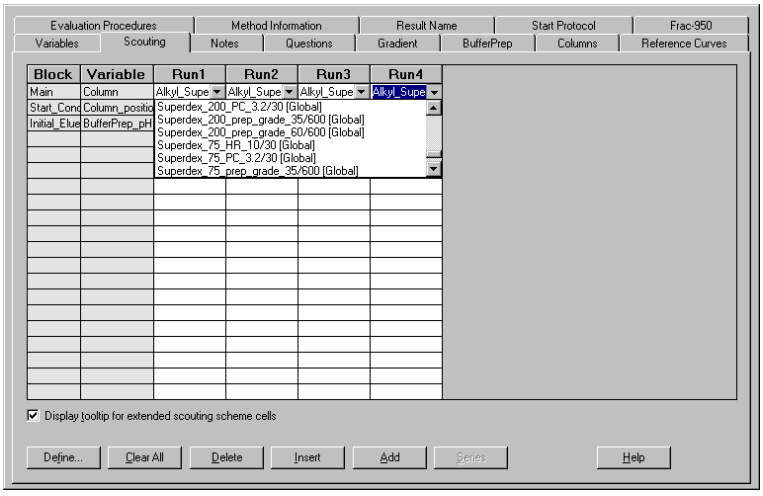


Fig 5-30. The Scouting tab in Run Set-up showing the Column drop-down menu.

4. When you have selected a column the **Column value update** dialogue is displayed.

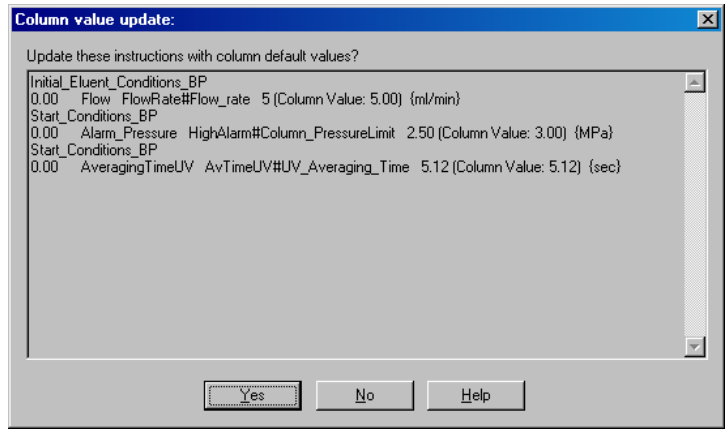


Fig 5-31. Column value update dialogue.

This dialogue asks if you want to update the method for the particular scouting run with the default values for the column parameters. The answer that you give to this dialogue has different consequences in terms of the scouting variables:

Yes The method for that scouting run is updated with variable parameter values for the selected column, consisting of UV averaging time, pressure limit, flow rate, etc. These parameter values are added to the scouting variables present on the **Scouting** tab. Note that the updated parameter values may differ from the values for the same variables in other scouting runs.

No No changes are made. The method retains the parameter values corresponding to the column that were either (i) originally selected during creation of the method, or (ii) included in an earlier version of the method on the **Scouting** tab where the default values for that column were accepted.

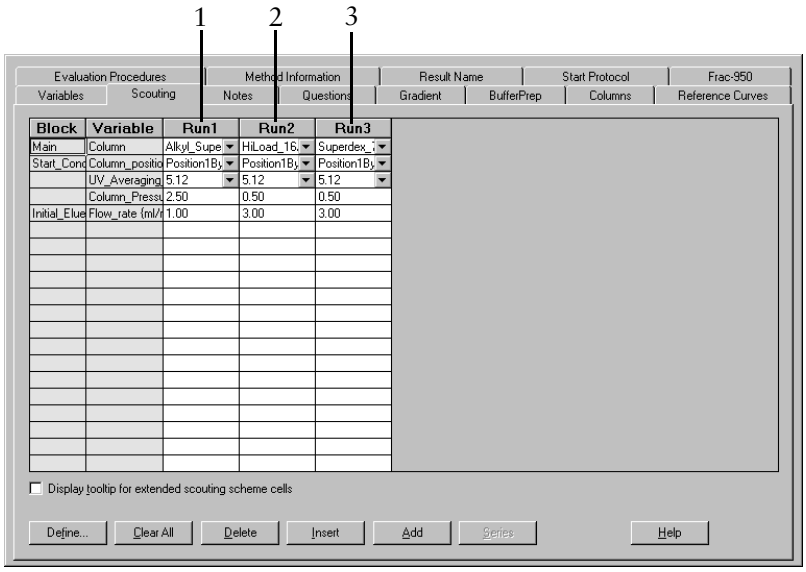


Fig 5-32. The Scouting tab in Run Set-up showing three different column types in three runs, 1-3. In Run 1 the default values for the column were accepted. In Run 2 the default values for the column were also accepted, so the variable values were updated for that run. In Run 3 the default values for the column were not accepted and thus the variable values are the same as those in the previous run.

Inserting scouting runs

To insert a new scouting run before an existing run, click on a run column on the **Scouting** tab and then click on **Insert**. The new run will inherit the variable values from the preceding run or from the default values in the method if the run is inserted at the beginning of the scouting series.

Adding scouting runs

To add a new scouting run click on **Add**. The new run inherits the variable values from the highest numbered run or from the default values in the method if the run is added at the beginning of the scouting series.

Excluding runs

In certain situations, it may be desirable to skip certain runs within the scouting scheme. To do so, click on the **Run** header in the **Scouting** tab. This will change the header from **Run** to **Excluded**.

Deleting scouting runs

To remove one run from the **Scouting** tab, click on any variable in the run and then click on **Delete**. Confirm that you want to delete the run.

To clear all runs, click on **Clear All** and confirm that you want to clear them all.

Start protocol for scouting runs

On the **Start Protocol** tab (see "Start Protocol" on page 96), you may choose whether to display the **Start Protocol** at the beginning of the first run only in a scouting series or at the beginning of every run. Displaying the **Start Protocol** at the beginning of the first run only allows unattended operation of the entire scouting series, but does not permit variable values and other settings to be adjusted at the beginning of each run. Displaying the start protocol at the beginning of every run requires that you go through the start protocol and then press the **Start** button to start each run.

Note: You must select **Scouting** in the **Start Protocol** tab in Run Set-up if you want to change the values for scouting variables before a run.

Converting a scouting run into a non-scouting run

To convert a scouting run into a non-scouting run, i.e. to have only the original method and variable values, click on the **Clear All** button and confirm the deletion of entries.

Factorial design

The contents of the **Scouting** tab can be copied (<Ctrl + C>) and pasted into a third-party factorial design program. Processed values can then be pasted (<Ctrl + V>) back into the **Scouting** tab.

5.5.3 Questions

Questions provide a means for entering structured run-specific information at the start of a run. Method Wizards and templates supplied with UNICORN are defined with a set of questions for sample, column and eluent identification. To define questions, open the **Questions** tab in Run Set-up.

Note: For questions to be shown in the start protocol, the **Questions** option must be checked in the Run Set-up **Start Protocol** tab.

Questions may have the following status:

- **Mandatory:** these questions must be answered before a method is started.

- **Authorised:** answers to these questions must be acknowledged by a user with **Confirm/Unlock** authorisation (see the UNICORN 4.0 Administration and Technical manual). The user's password must be given to acknowledge the answers.
- **Chromatogram:** these questions will be printed with the answers on the same page as the chromatogram if Diagram header is chosen in an evaluation report (see “Printing reports” on page 232).

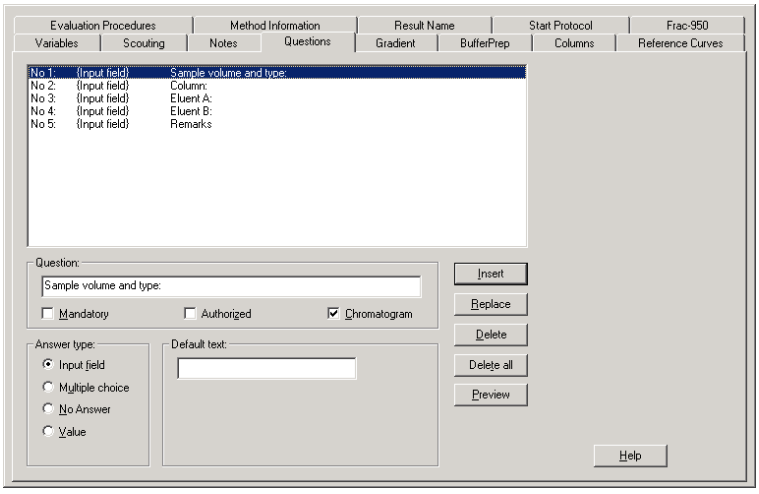


Fig 5-33. The Questions tab in Run Set-up

Questions may be defined to accept four types of answers:

- **Input field** accepts any alphanumerical input as the answer. Input field questions may have a default answer.

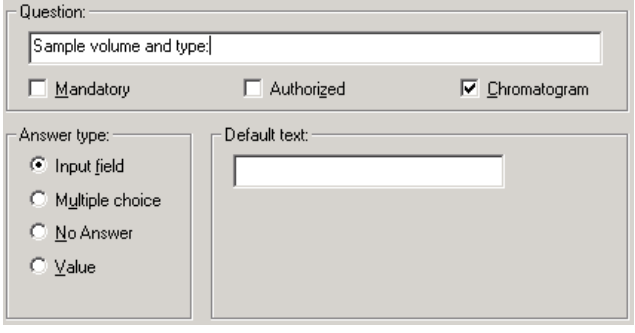


Fig 5-34. Options for Input field questions.

- **Multiple choice** allows the user to choose one of a defined set of answers. To allow a blank answer, enter a space in one of the pre-defined answers.

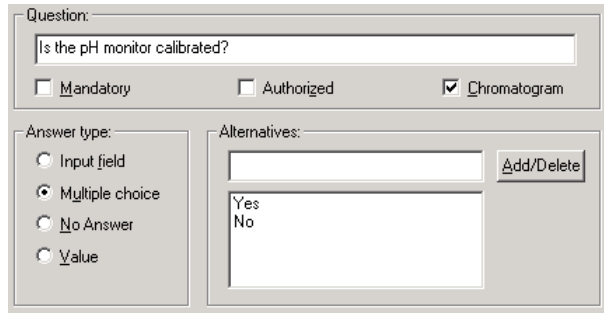


Fig 5-35. Options for Multiple choice questions.

- **No Answer** is not possible to answer. This kind of "question" may be to display important information or to split a question over more than one line (by setting all but the last line to **No answer**).

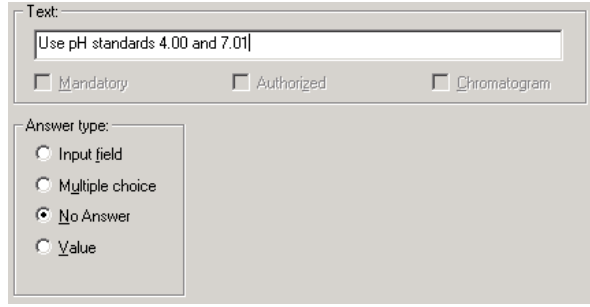


Fig 5-36. Options for No Answer questions.

- **Value** accepts only numerical answers. Value questions may have specified maximum and minimum limits, and may be defined to accept only integer values.

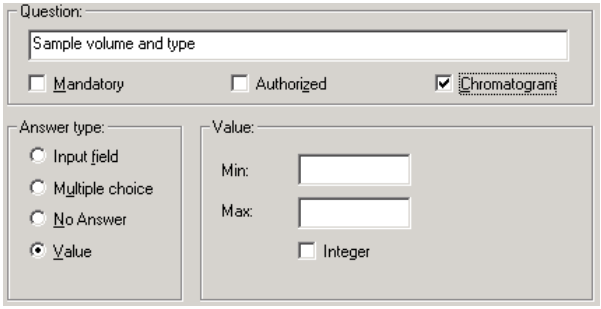


Fig 5-37. Options for Value questions.

On the **Questions** tab, press the **Preview** button to display the questions as they will appear when the method is run. (Alternative answers to multiple choice questions are not shown in this mode). From preview mode, press **Edit** to return to question editing mode.

Inserting a question

To insert a new question after an existing question:

1. Click on the existing question in the questions list.

2. Enter the text, status, type and answer for the new question as required. The **Answer type** panel determines what is displayed in the question definition field:

   - Input field questions: Enter a default answer if required.
   - Multiple choice questions: Click in the text field under **Alternatives**, enter the answer and click on **Add/Delete**. Repeat this procedure to add other alternatives. New alternatives are always added at the end of the list. To remove an alternative, mark the alternative in the scroll list and click on **Add/Delete**.
   - Value questions: Enter maximum and minimum limits. Check the **Integer** box if the question is to accept only whole numbers as answers.

3. Click on **Insert** to add the new question to the list.

   If the list is empty, the **Insert** operation creates the first question in the list.

Editing an existing question

To change the definition of an existing question, select the question to be changed. Change the text, status, type and answer as required and click on **Replace**.

Deleting a question

To remove a question, select the question and click on **Delete**. To remove all questions, click on **Delete All**.

5.5.4 Gradient

The Gradient tab provides a graphical overview of the block structure and eluent gradient in the current method. For scouting runs, click on the **Run** button to see the gradient for each run.

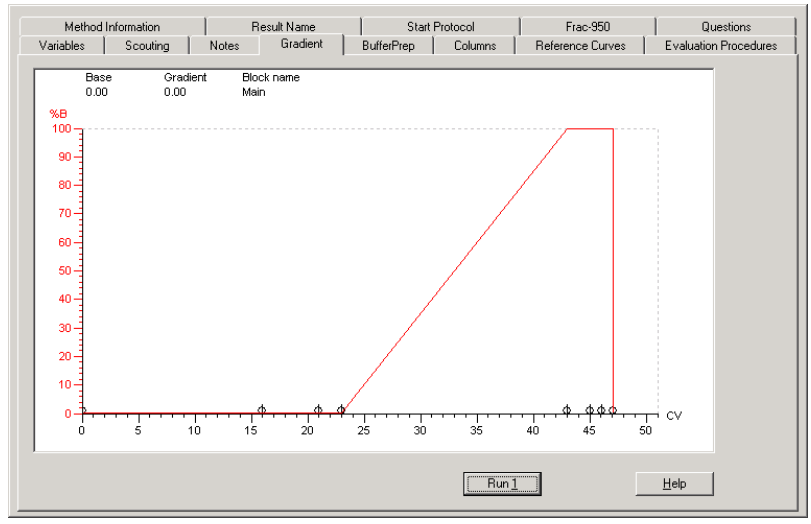


Fig 5-38. The Gradient tab in Run Set-up.

Vertical cursor line

A vertical cursor line can be dragged from the Y-axis with the mouse. As you drag the cursor line the current position is identified at the top of the page in terms of the block name, x-position in the currently displayed base and eluent concentration in % eluent B. The duration of variable length blocks are based on the default variable values.

The zoom function

To zoom-in on a selected region of the **Gradient** tab:

1. Press down and hold the left mouse button and drag a rectangle out on the screen to encompass the area to be viewed.

2. Release the mouse button. The display is now zoomed-in on the selected area.

3. Repeat the process for further magnification of selected areas.

You can reduce the scale of the zoom-in function in two ways, either:

- reverse each zoom-in action a step at a time by displaying the shortcut menu and selecting **Undo Zoom**, or,
- reverse all of the zoom in actions to the default scale setting by displaying the shortcut menu and selecting **Reset Zoom**.

Changing the base

You can select the base shown on the X-axis, either time, volume or column volumes. Make the appropriate selection from the shortcut menu option **Base type**. Changing the base for the display does not affect the base in the method instructions. You can therefore check how long a method will take simply by setting the axis scale to time, even if the method blocks are written in volume or column volume base.

Viewing hatch marks

You can display a hatched background in the **Gradient** tab by selecting **Hatch** from the shortcut menu.

5.5.5 Notes

Notes are descriptive comments that form part of the method documentation. There are four separate notes fields for method editing, start-up, run and evaluation respectively. Only the method notes can be edited from the Method Editor: the other notes are accessible at the respective stages in a run.

To view the method notes, open the **Notes** tab in the Run Set-up. Method templates are supplied with notes describing the system requirements for running the method. Read through these notes carefully before using a method.

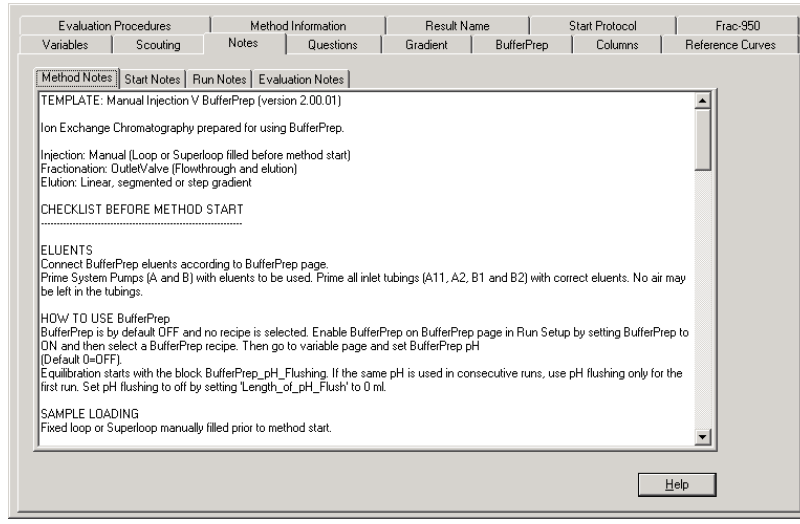

Evaluation Procedures
Method Information
Result Name
Start Protocol
Frac-950
Variables
Scouting
Notes
Questions
Gradient
BufferPrep
Columns
Reference Curves
Method Notes
Start Notes
Run Notes
Evaluation Notes
TEMPLATE: Manual Injection V BufferPrep (version 2.00.01)
Ion Exchange Chromatography prepared for using BufferPrep.
Injection: Manual (Loop or Superloop filled before method start)
Fractionation: OutletValve (Flowthrough and elution)
Elution: Linear, segmented or step gradient
CHECKLIST BEFORE METHOD START
ELUENTS
Connect BufferPrep eluents according to BufferPrep page.
Prime System Pumps (A and B) with eluents to be used. Prime all inlet tubings (A11, A2, B1 and B2) with correct eluents. No air may be left in the tubings.
HOW TO USE BufferPrep
BufferPrep is by default OFF and no recipe is selected. Enable BufferPrep on BufferPrep page in Run Setup by setting BufferPrep to ON and then select a BufferPrep recipe. Then go to variable page and set BufferPrep pH (Default 0=OFF).
Equilibration starts with the block BufferPrep_pH_Flushing. If the same pH is used in consecutive runs, use pH flushing only for the first run. Set pH flushing to off by setting 'Length_of_pH_Flush' to 0 ml.
SAMPLE LOADING
Fixed loop or Superloop manually filled prior to method start.
Help

Fig 5-39. The Notes tab in Run Set-up.

The notes are entered as free text and may be edited using standard Windows editing functions to edit the notes. Words wrap automatically at the end of the field.

Keystroke	*Action*
<Ctrl+X>	Cuts the marked text, saving it to the clipboard.
<Ctrl+C>	Copies the marked text to the clipboard.
<Ctrl+V>	Pastes the contents of the clipboard at the cursor.
<Delete>	Clears the marked text without saving it in the clipboard, or deletes the character to the right of the cursor if no text is marked.
<Backspace>	Clears the marked text without saving it in the clipboard, or deletes the character to the left of the cursor if no text is marked.

In the default method templates supplied with UNICORN, the method notes describe the system setup required by the method (e.g. eluent and

sample inlets, outlets, column connections and so on). We recommend that you use method notes for this purpose in your own methods, to provide documentation of the method requirements. Bear in mind that method notes are saved with the method and apply to all runs made with the method. Use the start or run notes for run-specific information. The date and time when the method was created and last edited are saved automatically in the method information, and need not be entered in the method notes.

5.5.6 Evaluation procedures

Evaluation procedures can be called automatically at the end of a method to evaluate and/or print the results. Method templates supplied with UNICORN include procedures named:

Integrate_and_Print which integrates the first UV curve in the chromatogram and prints out the results.

Print_Chromatogram which prints the chromatogram from the run with the scouting variables printed at the top.

User-defined procedures are created in the evaluation module and may be saved in method files (see "Automated evaluation procedures" on page 292). Procedures saved with one method file can be imported to another.

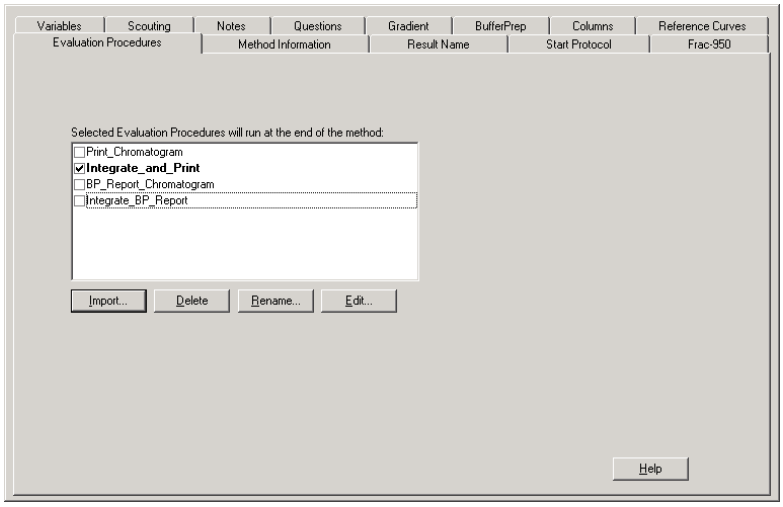


Fig 5-40. The Evaluation Procedures tab in Run Set-up.

Note 1: A procedure in a method will not be updated when a procedure with the same name is changed in Evaluation. The same applies to report formats saved in a procedure.

Note 2: If you use an evaluation procedure to print results automatically from a run controlled from a remote station in a network installation, the results will be printed on the printer currently set up on the local station, not on the remote station. If however you execute the procedure interactively from the evaluation module on the remote station, the results will be printed on the printer set up on the remote station where you are working.

Defining and viewing procedures

Evaluation procedures are normally defined in the Evaluation module. Procedures imported to a method can also be viewed and edited in the Method Editor; select the required procedure in the list and click on **Edit** (see "Editing an existing procedure" on page 294 for a description of how to edit evaluation procedures).

Note: Evaluation procedures which process chromatogram data rely on consistent identification of curves in the result file for correct operation. If you include evaluation procedures with a method, make sure that references to curves in the procedure will be valid when the procedure is executed at the end of the run (see "Automated evaluation procedures" on page 292 for more details).

Selecting procedures to run

The **Evaluation Procedures** tab lists all evaluation procedures associated with the method. Check the procedure(s) that are to be executed at the end of the run. The procedures will be executed in the order they appear in the list.

Importing procedures

To import an evaluation procedure:

1. Select the **Evaluation Procedures** tab and click on **Import.**
2. Choose a procedure from the **Select list**. You can also choose to import a procedure from another method. Select a method to show the procedures stored in the method. If you have chosen a method, click on **Evaluation Procedures** to return to the complete list.
3. If desired, change the procedure name in the **Import as** field.
4. Click on **Import**.

   The dialogue remains open until you click on **Close**, so that you can continue to add procedures from the same or different method files.

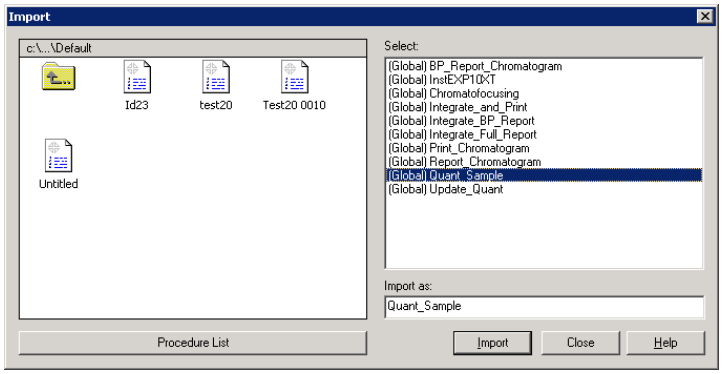


Fig 5-41. The Import procedures dialogue.

Deleting procedures

To remove one or more procedures from the method:

1. Select the **Evaluation Procedures** tab and check the procedure(s) to be deleted.

2. Click on **Delete** and confirm the deletion.

Note: Procedures that you delete from the method are immediately removed from the method file.

Renaming procedures

To rename a procedure in a method:

1. Select the **Evaluation Procedures** tab and click on **Rename**.

2. Select a procedure from the list and change the name in the **Rename item to** field.

3. Click on **Rename**.

The dialogue remains open until you click on **Close**, so that you can rename more than one procedure without closing the dialogue.

Editing procedures

To edit a procedure in a method:

1. Select a procedure on the **Evaluation Procedures** tab and click on **Edit**.

2. Edit the procedure as described on page 294.

3. Choose **File:Exit** from the procedure editor menu. (**File:Save** is not available in the procedure editor window when you edit procedures in a method. Changes are saved automatically when you close the procedure editor).

Note: Report formats in procedures cannot be edited or viewed.

5.5.7 Reference curves

Reference curves are curves from existing result files which you can display in the curve window of System Control during a run. You can include up to 5 reference curves in a method. You choose which curves to display during the run with the **View:Curve contents** command in System Control (see "Curves" on page 149). Reference curves are only displayed during the run and the Y-axis can be made active for the reference curve by clicking on its legend. Reference curves are not saved in the result file.

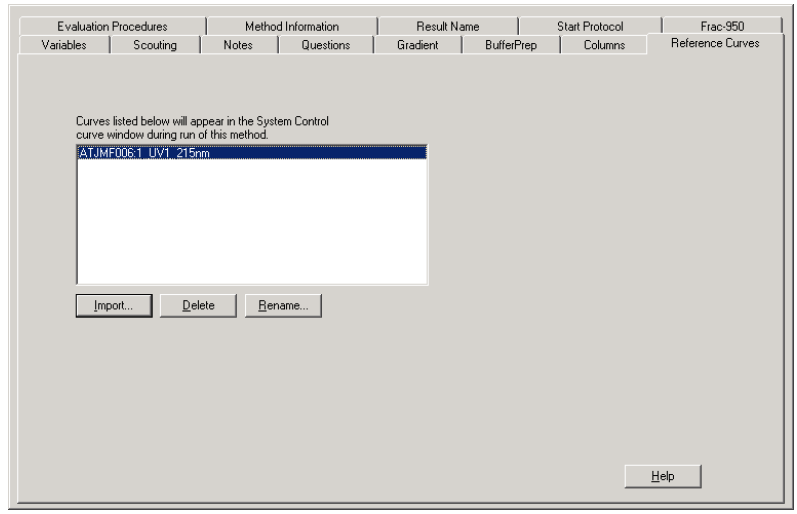


Fig 5-42. The Reference Curve tab in Run Set-up.

Adding curves

To add a reference curve from a result file:

1. Select the **Reference Curves** tab and click on **Import**.

2. Choose the result file containing the curve to be added and select the curve from the **Select** list.

3. If desired, change the curve name in the **Import as** field.

4. Click on **Import**.

The dialogue remains open until you click on **Close**, so that you can continue to add curves from the same or different result files.

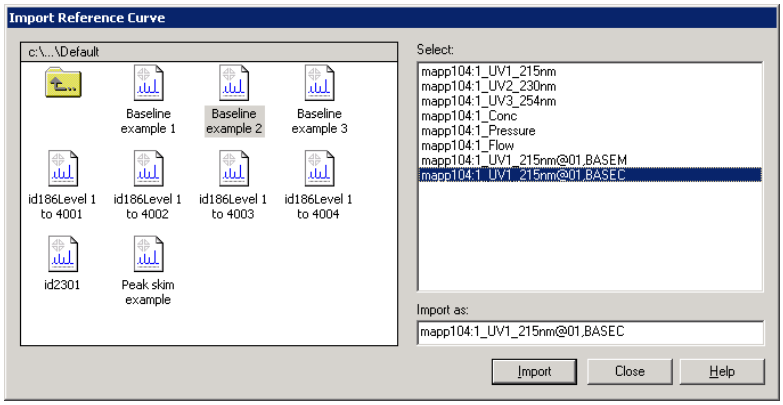


Fig 5-43. The Import Reference Curve dialogue.

Deleting curves

To remove one or more reference curves from the method:

1. Select the **Reference Curves** tab and select curve(s) from the list.

2. Click on **Delete** and confirm the action.

Note: Deleting curves from the method does not affect the curves in the result file from which they were imported.

Renaming curves

To rename a reference curve in a method:

1. Select the **Reference Curves** tab and click on **Rename**.

2. Select a curve from the list and change the name in the **Rename item to** field.

3. Click on **Rename**.

The dialogue remains open until you click on **Close**, so that you can rename more than one curve without closing the dialogue.

5.5.8 Columns

You can look at the parameters of the column selected for your method by clicking on the Column tab. The column parameters will be displayed in the **Column Data** field. If you are performing scouting runs with different columns, all of these will be listed. Select the appropriate column to display the parameters.

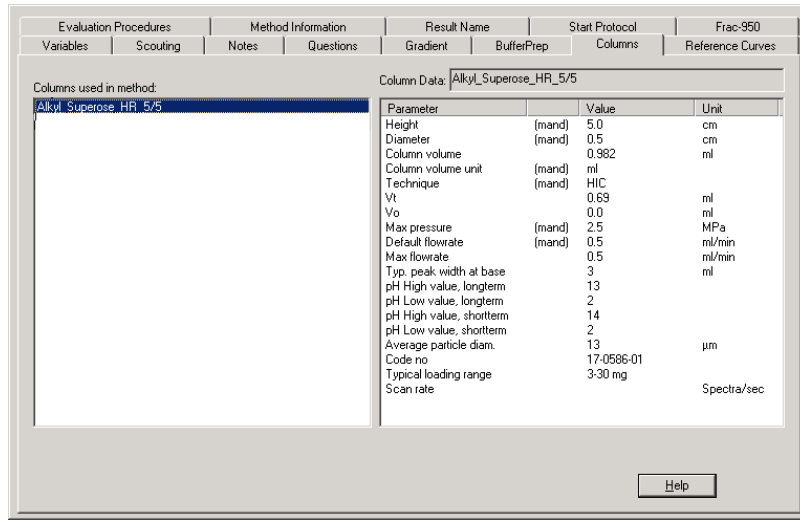


Fig 5-44. The Columns tab in Run Set-up.

5.5.9 BufferPrep

BufferPrep is only available for some ÄKTAdesign systems. BufferPrep allows a buffer of any pH and salt concentration to be prepared on-line from 4 stock solutions. This removes the need to manually prepare new buffers every time the pH needs to be changed. Linear and step salt gradients can be run and pH can be used as a variable scouting parameter. BufferPrep is optimised for cation and anion exchange chromatography. It can be used for gel filtration, but not for other chromatographic techniques. For a complete description of BufferPrep, see the User Manual for ÄKTAdesign systems.

Note: If a suitable template is not available you can create a BufferPrep method by selecting the BufferPrep tab, clicking on the **BufferPrep ON** radio button. The instruction **BufferPrep_pH** must be available at breakpoint zero at the beginning of the method. The method must not contain the instructions **PumpAInlet** or **PumpBInlet**.

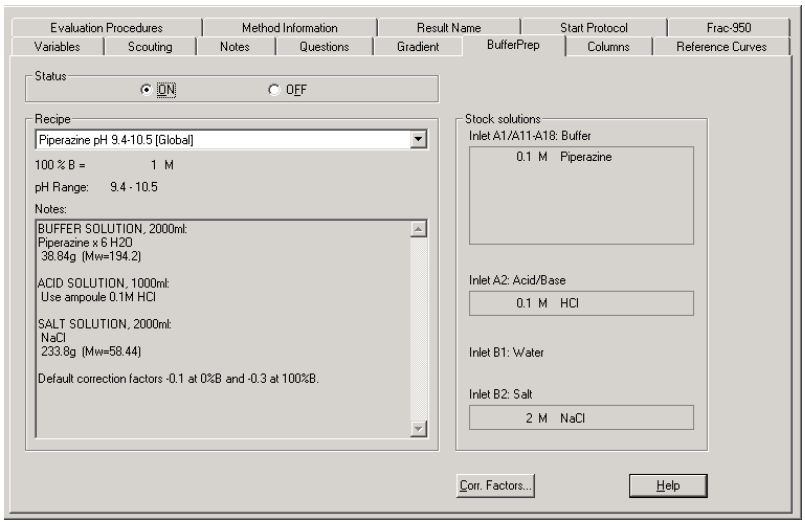


Fig 5-45. The BufferPrep tab in Run Set-up.

1. On the **BufferPrep** tab select a **Recipe** from the drop-down list box, either AIEX or CIEX, which are recipes covering a broad pH range. Otherwise select one of the single buffer recipes for more narrow pH ranges. All information relevant to the selected recipe will be displayed on the tab.

2. Prepare the required stock solutions. The solutions and the inlets to which they should be connected are displayed to the right of the page. Accuracy of preparation is essential. The four stock solutions consist of:

   i. A mix of buffering components. There can be up to five different buffering components enabling a broad pH range to be covered.

   ii. An acid (HCl) or base (NaOH) for pH on-line titration.

   iii. Distilled water.

   iv. An inert salt (e.g. NaCl) for salt gradient formation.

3. Select the **Variables** tab. Set the required pH for the run in the variable **BufferPrep_pH**.

Alternatively, if you want to perform pH scouting, click on the **Scouting** tab and select **BufferPrep_pH** as a scouting variable and enter the pH values for the different runs.

The recipe saved in the method (the one selected on the **BufferPrep** tab) cannot be edited although fine tuning is possible (see below). However, the recipes in the list of all BufferPrep recipes (**Edit:BufferPrep Recipes**), can be edited (see "Editing a BufferPrep recipe" on page 137). New recipes can also be created (see "Creating a BufferPrep recipe" on page 133).

Fine tuning with correction factors

In order to obtain high pH accuracy, the recipe can be fine tuned around a specific pH by setting correction factors. Run BufferPrep manually at 0% and 100% and change correction factors in the method as follows:

1. Select the **Recipe** under group **Other** in **System Control:Manual**. Click on **Execute**. Set the pH in the instruction **BufferPrep_pH** in group **Pump**. Click on **Execute**. Set the flow rate to be used during the run in instruction **Flow**. Click on **Execute**.

2. Check the pH reading when stable. Allow at least 30 ml of eluent to pass through before expecting a steady pH reading.

3. Change to 100% B by setting the **Gradient** instruction in **Manual:Pump** to 100% for **Target** and 0 for **Length**. Click on **Execute**.

4. Check the pH reading when stable at 100% B.

5. If the readings are acceptable at both 0% and 100%, the correction factors do not need to be changed.

6. If the readings are not acceptable, click on **Corr. Factors** in the BufferPrep tab in the method.

7. Enter the deviation at 0% and 100%. (E.g.: if the pH is set to 7.0 and the actual pH is 7.1 enter 0.1. Enter -0.1 if the pH is 6.9).

Note: If correction factors already exist, the measured pH deviation should be added to the old factors.

8. Save the method.

Note: When changing the correction factors for the recipe selected in the method, the recipe with the same name in the list of all BufferPrep recipes is not affected. The changes will only apply in the specific method.

For changing the default correction factors in a recipe not saved in a method, see “Editing a BufferPrep recipe” on page 137.

5.5.10 Method Information

The **Method Information** tab displays information about the method, such as method name, target system, and date of last change. Strategy information includes, strategy name, date and size. The **Strategy Notes** button displays a list for which systems, programs and file versions the strategy is designed for.

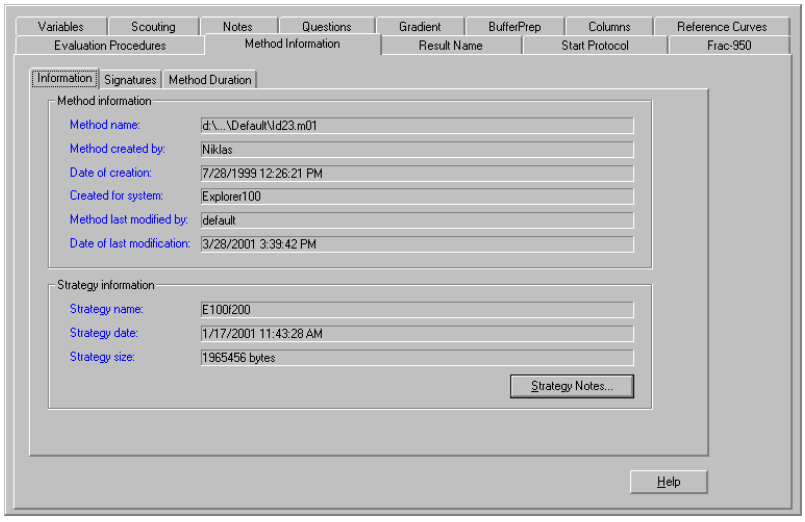


Fig 5-46. The Method Information tab in Run Set-up.

There are also tabs for **Signatures** (see “Signing the method” on page 39) associated with the method, as well as **Method Duration**.

The **Method Duration** presents the estimated total time and buffer volume required for the method. If the method includes a scouting scheme, click on the **Run ‘x’** button to see estimated values for the times and volumes for the different scouting runs. The **Method Information** tab is for information only and cannot be edited.

5.5.11 Result Name

Use the **Result Name** tab to specify how the result files will be named for the results of a run, and where the result file will be saved. You can also specify the name of the special scouting folder where results from scouting runs will be stored.

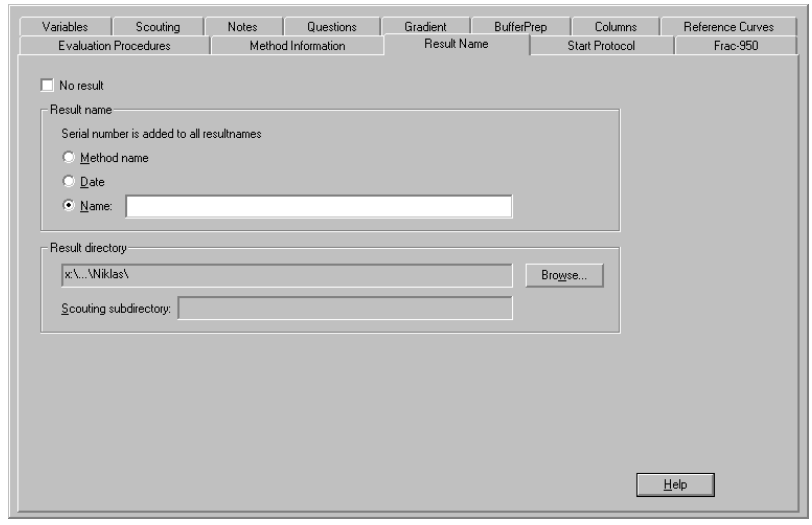


Fig 5-47. The Result Name tab in Run Set-up.

The result file name is constructed by adding a 3-digit serial number to one of the base options listed below. The serial number is incremented automatically each time the method is run:

- the **Method name** plus a 3-digit serial number
- the **Date** of the run (in an 8-digit format determined by the country setting in Windows NT) plus a 3-digit serial number
- a freely specified **Name** (within the file naming restrictions in the operating system) plus a 3-digit serial number

If the result file folder already contains files with the same file name base, the serial number is incremented automatically. For scouting runs, the 3-digit serial number will be the number of the executed run column in the scouting scheme.

By default, result files are stored in the home folder of the user who starts the run. To change the folder where the result file will be stored, press the **Browse** button, double-click on the required folder icon and press **Close**. If the run will contain information that is not important, you can save file space by checking the **No result** checkbox, thereby storing the result in the Temporary folder (named Manual Runs, where only the latest 10 result files are saved).

Note: The result name may be specified as changeable in the Start protocol (see below). In that case, the specification in the **Result** tab serves to generate the suggested result name, which may be changed at the start of the run.

Scouting results will be saved in a special folder on the result file path. Enter a name for the folder in the **Scouting folder** field. Each time the scouting method is run, a new folder will be created with the name and a serial number (e.g. entering IEXSC will create folders IEXSC 1, IEXSC 2 etc.).

5.5.12 Frac-950

User-defined options for Frac-950 are selected on the Frac-950 tab. The **Frac-950** tab allows the user to choose rack type and the fractionation order.

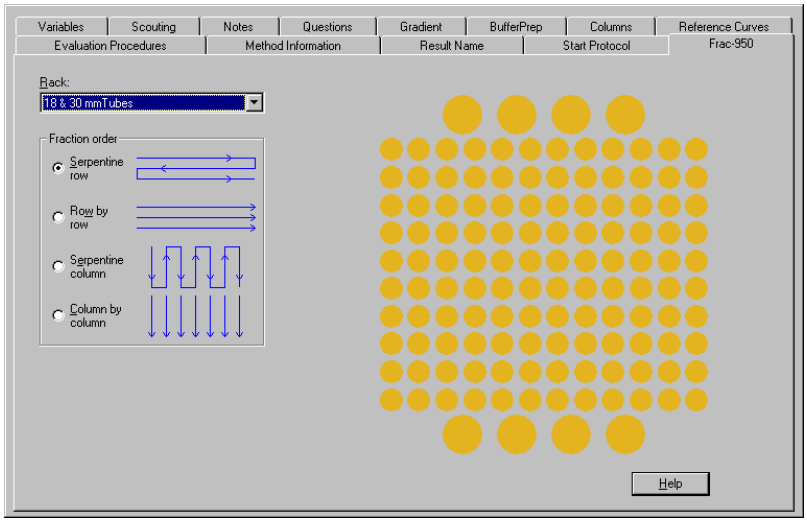


Fig 5-48. The Frac-950 tab.

Rack type

Begin the fractionation set up by choosing the appropriate rack type from the **Rack** drop-down menu.

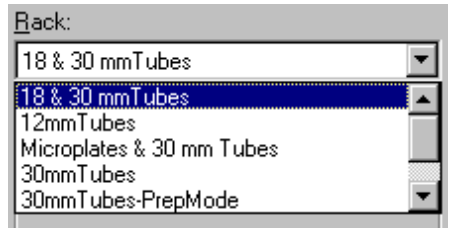


Fig 5-49. The Rack drop down menu within the Frac-950 tab.

Fraction order

Next, use the **Fraction order** radio buttons to choose the appropriate order for fractionation.

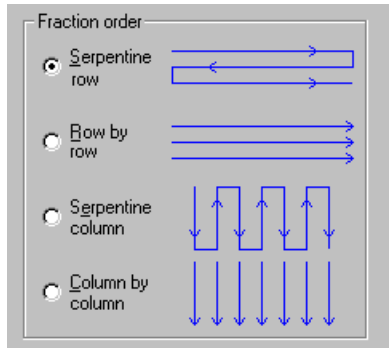


Fig 5-50. The Fraction order radio buttons.

Note: In System Control, for manual runs, the **Frac-950** tab cannot be used in Documentation. Instead, use the manual fractionation instructions, starting with Man_ .

Last tube

The last tube position can only be selected from within **Start Protocol** before starting a run. Click the **Define** box within the **Last tube** panel, and then move the cursor over the appropriate tube (circle) within the tube matrix and click again.

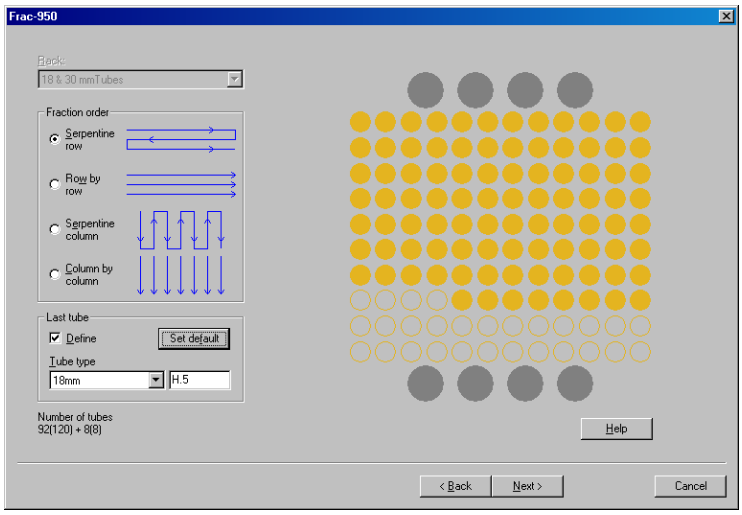


Fig 5-51. The Frac-950 tab in Start Protocol, illustrating the Last tube function.

If the fractionation process attempts to proceed beyond this position during a run, an alarm will be executed. The lower right box within the **Last tube** panel shows the currently selected last tube.

If a new last tube has been chosen, but the user wishes to return to the default last tube position, simply click on the **Set default** button. This will automatically set the last tube to its default position.

Note: When using different sized tubes (racks: 18mm tubes or Microplates) the last tube can be set for both tube sizes. Use the **Tube type** drop down menu to choose the desired tube size, and then follow the procedures outline above to select the last tube.

Note that the total number of tubes sampled may differ according to which **Fraction order** has been chosen.The **Number of tubes** equation in the bottom left corner of the **Frac-950** tab shows the current number of available tubes chosen for fractionation, followed by the total possible number in parentheses. For example, in the figure above, 92 of the total 120 small tubes, and all 8 of the large tubes will be available for fractionation.

5.5.13 Start Protocol

The Start Protocol tab determines which items of the Run Set-up are displayed (and may in appropriate cases be altered) at the start of a run. Click on the **Start Protocol** tab and check the items that are to be displayed.

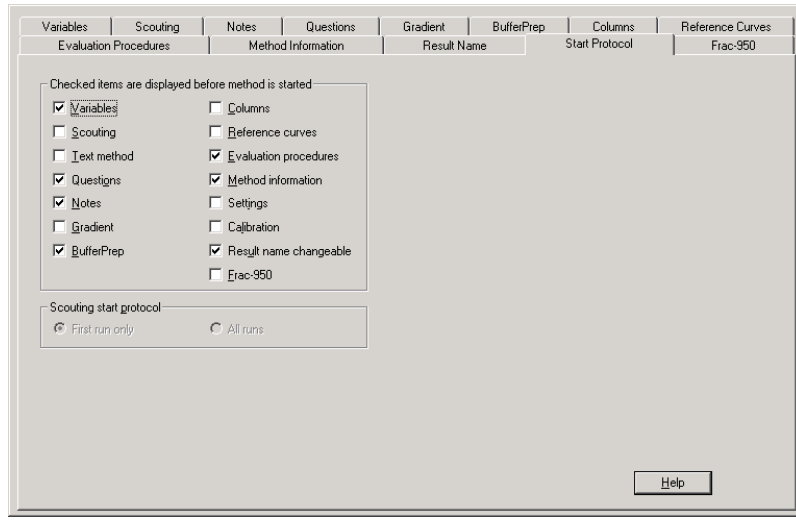


Fig 5-52. The Start Protocol tab in Run Set-up.

Variables

If this box is checked, values for method variables will be displayed and can be changed at the start of the run. These values will override the default values for the particular run, and will be saved in the result file. The default values stored in the method are however not affected. Variables defined for scouting cannot be changed in the **Variables** tab.

If the **Variables** box is not checked, the run will be executed with default values for all variables as defined in the method, unless scouting is used (see below).

Scouting

If this box is checked, the scouting scheme will be displayed and can be changed at the start of the run. Changes will override the default settings and values for the particular run, and will be saved in the result file.

Note: Runs may also be excluded or included during a run using the System Control documentation.

Note: Variable values in the scouting scheme will override any values in the **Variables** tab.

Text Method

Displays the method instructions. Double-click on a Block instruction, as denoted by the blue square and '+' mark, to display or hide the instructions in the called block (see "Viewing instructions" on page 57). Method instructions cannot be changed from this display.

Questions

If this box is checked, questions defined in the method will be displayed at the start of the run.Since the answers to questions can form an important part of the run documentation, you are recommended always to check the **Questions** box.

Notes

If this box is checked, the **Notes** tab will be displayed at the start of the run. You can enter notes in the **Start notes** tab but not in any of the other tabs. You can use the scroll bar if necessary to read notes in the **Method notes** tab.

Gradient

If this box is checked, you can view the gradient at the start of the run.

BufferPrep (Only available to some systems)

If this box is checked the recipe selected in the method will be displayed at the start of the run. You cannot change recipe at the start of a run.

Columns

If this box is checked, you can view the available column definitions (see "Columns" on page 88). The column definition used in the method run is selected with the Base instruction, and may be changed at the beginning of the run on the **Variables** tab if the columns parameter is defined as a variable (see "Variables" on page 70).

Reference curves

If this box is checked, the reference curves associated with the method will be displayed at the start of the run. You can add, delete and rename curves at the start of the method. All curves in the list can be displayed in System Control during the run.

Evaluation procedures

If this box is checked, the evaluation procedures set to execute at the end of the method will be displayed at the start of the run. You can change the choice of procedures to execute, but you cannot add or

remove procedures. (Procedures are stored as part of the method file, which cannot be changed at the start of the run).

Method information

If this box is checked, the method information (including creator, target system, strategy information and date and time of creation and latest change) will be displayed at the start of the run. You cannot edit the method information. There are also tabs for **Signatures** associated with the method, as well as **Method Duration**.

The **Method Information** tab is for information only and cannot be edited.

Settings

If this box is checked, the settings (including alarms, monitors and curve configuration) will be displayed for information at the start of the run.

To change settings, use the **System:Settings** command in System Control before starting the run (see the UNICORN 4.0 Administration and Technical manual).

Calibration

If this box is checked, the monitor calibration settings will be displayed at the start of the run. If not satisfied with the calibration, you can still calibrate the monitors before the run is started by using the **System:Calibrate** command in System Control.

Result name changeable

If this box is checked, you can change the result name when the run is started. Click on the **Browse** button to change the result folder.

If the **Result name changeable** box is not checked, the result name will still be displayed, but neither the name nor the folder can be changed.

Frac-950

If this box is checked, you can view and change any of the Frac-950 setup parameters.

Scouting start protocol

Check the appropriate option for the Scouting start protocol display in scouting runs. If you check **First run only**, parameters for the scouting runs can be adjusted at the beginning of the first run only, and the runs will thereafter be performed automatically without operator intervention. If you check **All runs**, the Scouting start protocol will be displayed at the beginning of each run in the scouting scheme.

5.6 Saving the method

5.6.1 Saving a method

Click on the **Save** icon or choose **File:Save** to save the method. If you want to save a copy of the method under a new name, choose **File:Save As** and enter the details as described below.

1. Enter a name for the method. Method names may contain letters (A-Z) and digits (0-9). The case of letters is not significant. The method name must be unique for the chosen system within the folder (see steps 2 and 3 below).

2. By default, the method will be saved in your home folder. To change the folder, double-click on the appropriate folder icon in the Methods panel.

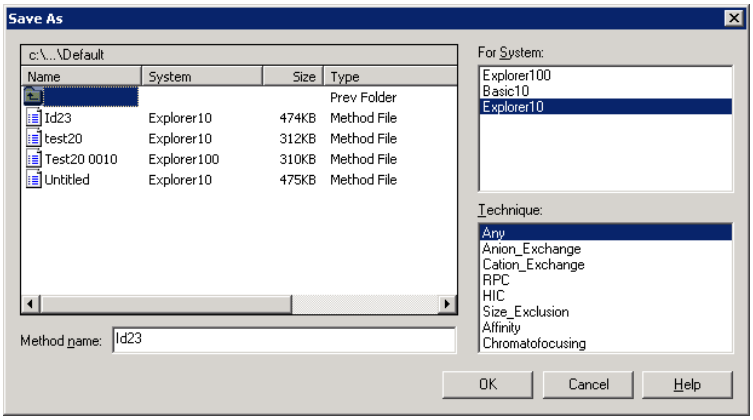


Fig 5-53. Save As dialogue for saving a method.

3. If you have more than one system available, choose the system for which the method is intended. The method can only be run on the strategy for which it is saved. Remember that different systems may have different configurations and control capabilities.

4. Click on **OK**.

5.6.2 Saving as a template

You can save the method as a template if you have **Edit global lists** authorisation (see the UNICORN 4.0 Administration and Technical Manual).

1. Choose **File:Save as Template**. The **Save As Template** dialogue is displayed.

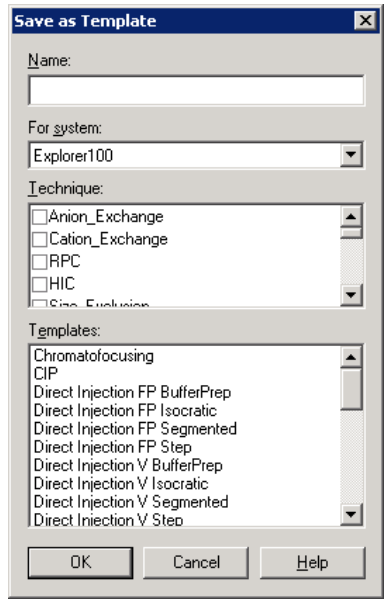


Fig 5-54. Save as Template dialogue.

2. Enter a name for the template in the **Name** field, or choose an existing template name from the list.

3. Choose the system for which the template is intended in the **For system** field.

4. Check the appropriate **Technique** from the list.

5. The **Templates** field lists the templates currently available within the chosen system, and is useful for reviewing which names are in use.

6. Click on **OK**.

Note: The templates for each system are common for all users. Be restrictive in saving methods as templates. We recommend that only methods that are useful for all users are saved as templates.

5.6.3 Deleting a template

You can delete templates if you have **Edit global lists** authorisation.

1. Choose **Edit:Delete template**.

2. Select the system and the template to delete, and click on **Delete**.
3. Confirm the action.

Note: The templates for each system are common for all users. Be restrictive in deleting templates.

5.7 Printing the method

You can print a copy of the method including items from the method documentation (Run Set-up) and the Text instructions window.

1. In the Method Editor select **File:Print** or click on the **Print** icon.

   The **Print** dialogue is displayed. Only items available from the Method Editor can be selected.

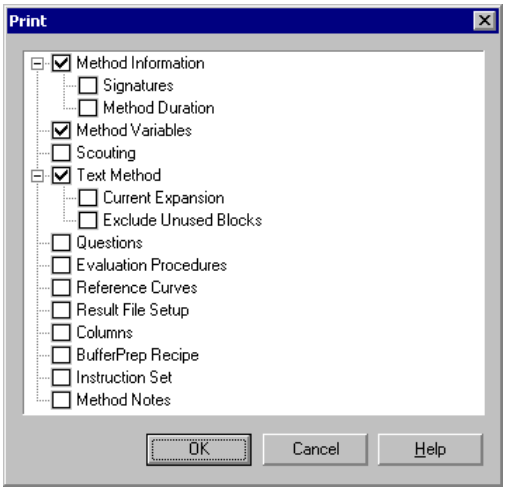


Fig 5-55. Print dialogue within the Text instruction view.

Note1: If you would like access to the **Current Expansion** print option, you must select the print command from the Text instructions view.

Note2: By checking the **Text method** option all instructions will be printed including those in the unused blocks. If you check the **Print current expansion** option the method will be printed according to the current expansion in the Text instructions window. If you check the **Exclude unused blocks** option only those blocks used in the method will be printed.

2. Check the options that you want to print out.

3. Click on **OK** to print.

5.8 Exporting Methods

This dialogue should ideally be opened while in Text Instructions mode. To export a method select **File:Export:Method** to access the **Export Method** dialogue.

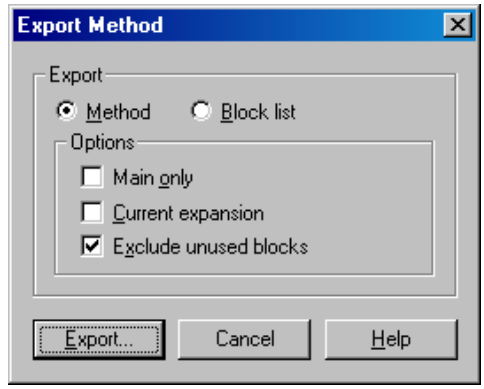


Fig 5-56. The Export Method dialogue.

Select whether the current method should be exported as a **Method** or as a **Block list**. Check the appropriate **Option** boxes to define the level of detail in the information.

Select **Export** to prompt the **Export Method to file** dialogue. Enter a file name and select the target drive and folder. Click on **Save**.

5.9 Exporting Run Set-up

This dialogue is used to export information in Run Set-up. Select **File:Export:Run Set-up** to access the **Export Run Set-up** dialogue.

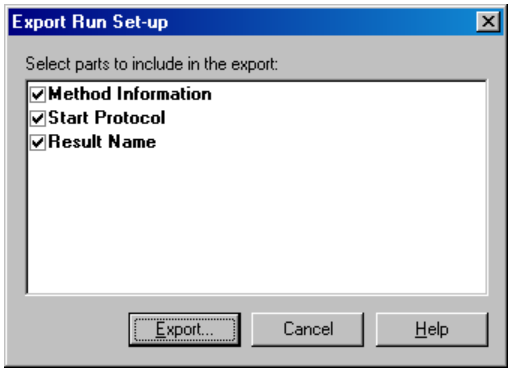


Fig 5-57. The Export Run Set-up dialogue

Check the boxes to select the parts of the Run Set-up to be exported. Select **Export** to prompt the **Export** dialogue. Enter a file name and select the target drive and folder. Click on **Save**.

5.10 How to use selected unconditional method instructions

This section provides recommendations for how to use some common programming features in UNICORN methods.

5.10.1 Base instruction

Every method block must start with a **Base** instruction, defining the base for calculating breakpoints. The base may be volume (ml or l depending on the scale defined in the system strategy), time (min), or column volume CV (defined as a numerical value or taken from the column definition). For all blocks other than the main block, the base may also be defined as **SameAsMain**, which means that the block will inherit the base defined in the main block. Different blocks may use different bases.

Use the base which most closely suits the purpose of the block. Column volume is recommended as the base for most steps in a run. In some situations, it may be more suitable to use time or volume base for individual blocks.

Be careful when changing the base for an existing method. Changing between time and volume base can affect the relative duration of steps in the method if different steps use different flow rates.

Note: For method blocks which use a volume or column volume base, make sure that the flow rate is not zero. Volume breakpoints are calculated from the flow rate of the pump, and the method will not progress if the flow rate is zero.

The parameters for the **Base** instruction differ slightly according to whether a named column definition is used:

Column parameter: Any

If the **Column** parameter in the **Base** instruction is set to **Any** and the **Base** parameter is set to **CV**, the column volume is set numerically by the **Volume** parameter. The column volume may be defined as a variable, allowing the scale of the run to be decided when the method is actually run.

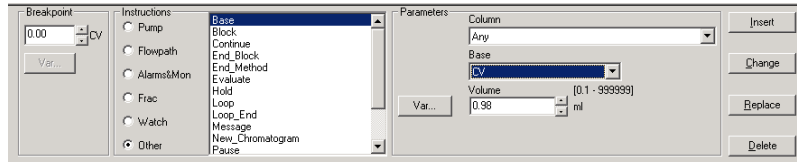


Fig 5-58. With column volume selected for the Base instruction and Any selected for the Column parameter, the column volume is entered numerically and may be defined as a variable.

Column parameter: named column

If a named column is selected for the **Column** parameter in the **Base** instruction, the volume specified in the selected column definition will automatically be used for column volume in the method block. The column volume for base CV cannot then be changed in the instruction or defined as a variable. The **Column** parameter can however be defined as a variable (recommended). Choosing a column definition also enables linear flow rate and column performance calculations.

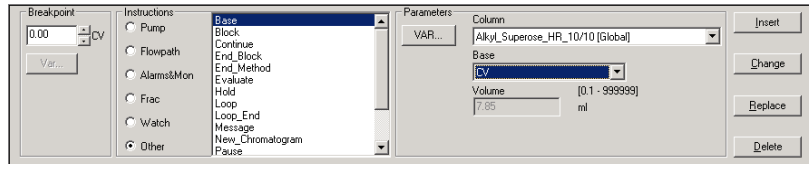


Fig 5-59. Using a named column definition in the Base instruction. The column volume is taken from the column definition and cannot be changed in the instruction. The column name can be defined as a variable.

In cases where a template (including Wizard-generated methods) and column are chosen, it is easy to select other columns for that template in the **Variables** tab. To select a column for a method when the method is not selected from a template:

1. Define new columns in the **Columns** list if required (see "Adding a new column" on page 128).
2. In Text instruction mode, mark the **Base** instruction in the method (the first instruction).
3. For the **Column** parameter in the Instruction box, choose the required column from the drop-down menu.
4. Define the **Column** parameter as a variable (recommended). This will make it easy to change the column selection for different runs.
5. Click on **Change** in the Text instruction box.

Note 1: A selected column definition applies locally within the block for which it is selected, and is not transferred to other blocks. We strongly recommend that the column definition is selected for the main block.

Note 2: A column definition can be chosen and defined as a variable even if the base for the block is set to volume or time. Parameters in the column definition will then be used for linear flow rate and column performance calculations.

Note 3: If you want parameters to be updated when you change the column, you must define flow, pressure and averaging time as variables.

The Methodbase instruction

Volume or column volume base is calculated from the flow rate of the SystemPump or the SamplePump, selected with the instruction **Methodbase**. If no **Methodbase** instruction is included in the method, the default setting **SystemPump** will be used.

5.10.2 Instructions at the same breakpoint

Instructions placed at the same breakpoint in a block are executed simultaneously, with the exception of successive **Block** instructions which are executed in the sequence in which they are written. This can have important consequences in some situations. For example, the instruction sequence:

```
...
0.00    Block WASH
0.00    FractionCollector 5{ml}
0.00    Block ELUTE
...
```

will start the fraction collector when the **Wash** block is completed.

5.10.3 Block and method length

The length of a block is determined by the breakpoint of the last instruction in the block. A block in which all breakpoints are at 0 will take no time or volume during a run, e.g.

```
   (Initial_Eluent_Conditions_BP)
   0.00  Base SameAsMain
   0.00  BufferPrep_pH (7)#BufferPrep_pH
   0.00  BufferValveA1 (A11)#Buffer_Inlet
   0.00  Flow (5)#Flow_rate {ml/min}
   0.00  Gradient (0.00)#Start_ConcB {%B}, 0.00 {base}
   0.00  End_block
0.00  End_block
```

To extend the length of a block without performing any other operation, set the breakpoint of the **End_block** instruction appropriately, e.g.

```
(Equilibration)
0.00     Base SameAsMain
4.00     End_block
```

During a run, the overall time or volume is determined by the sum of the block lengths.

Note: The length of the main block does *not* indicate the overall length of the method (the main block often consists only of calls to other blocks and has zero length). The method length can be checked in the Gradient window of System Control.

Log format

The cumulative method time or volume can be viewed in Method Editor by viewing the **Log Format**.

To view the cumulative time or volume for a method select **View:Log Format** or click on the **Log Format** icon. The **Log Format** dialogue is displayed.

The **Log Format** dialogue displays the cumulative time or volume for the current method. If the method is a scouting run, click on the **Run** button to move between runs.

Note: The cumulative time/volume is an approximation and does not take into account time or volume for **Watch** blocks, **Wash** commands, or programmed **Hold**.

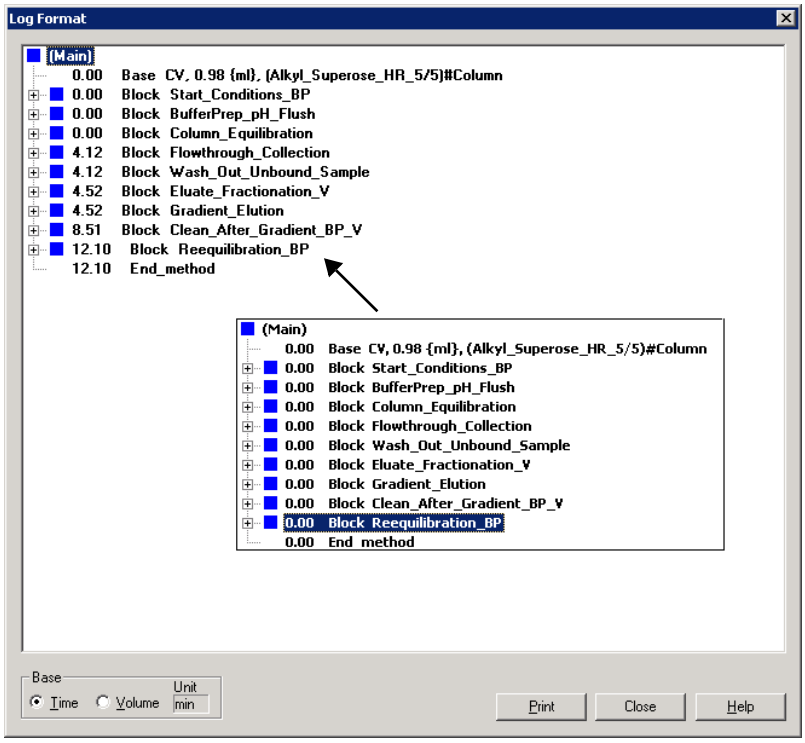


Fig 5-60. Log Format dialogue displaying the cumulative time for a method (block breakpoints shown in rectangle with arrow).

The concept of block time or volume versus cumulative time or volume is illustrated in the following table:

Cumulative time/vol	Block time/vol	Instruction	
		(Main)	
0	0	0.00	Block EQUILIBRATE(Equilibrate)
0	0	0.00	Base SameAsMain
4	4	4.00	End_block
4	0	0.00	Block SAMPLE_INJECT(Sample_inject)
4	0	0.00	Base SameAsMain
4	0	...	
9	5	5.00	End_block
9	0	0.00	Block GRADIENT
		(Gradient)	
9	0	0.00	Base SameAsMain
9	0	0.00	Gradient 100,20
29	20	20.00	End_block

Depending on how conditional calls are used (see "How to use selected conditional method instructions" on page 112), the overall method time or volume may vary according to watch events during the run.

5.10.4 Messages and Set_mark

Messages

Use messages to inform the operator of the progress of the run. It is a good idea to issue messages at critical points in the method, e.g. when **Watch** instructions are used for conditional events. The example block below starts the fraction collector and informs the operator:

```
(Start_frac)
0.00 Base SameAsMain
0.00 Message "Fraction collection started",Screen
0.00 FractionCollector 5{ml}
0.00 End_block
```

Messages which are set to **Screen** will be displayed on the screen during a run, and will remain there until acknowledged by the operator. Messages can also be set to **Noscreen**: these will be recorded in the run log but not displayed on the screen.

Set_mark

Other text messages can be inserted into the chromatogram at set points using the **Set_mark** instruction. The **Set_mark** instruction is also a convenient way of inserting a note into both the logbook and the chromatogram during a run. This contrasts from **Message**, which is displayed on the screen and entered into the logbook only.

Set_mark can be used to insert manual notes, for example when a problem occurs in the run. The instruction can also be incorporated into a method as shown in the following example where **Set_mark** is used to highlight the start and end of peak fractionation in a method.

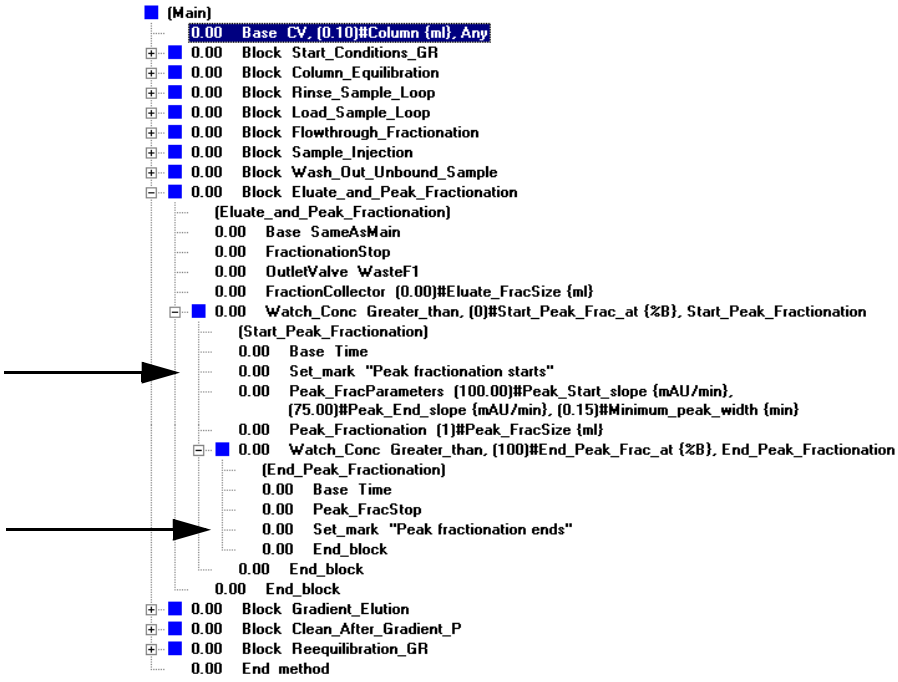


Fig 5-61. A method containing the Set_mark instruction (see arrows) to show the start and end of peak fractionation.

5.10.5 Pausing a method

A method can be programmed to pause at critical points. There are three instructions for this purpose:

Hold suspends execution of the method, but continues to pump eluent at the current flow rate and concentration settings. This instruction is useful e.g. for giving the operator time to load a sample loop:

```
(Sample_injection)
0.00 Base SameAsMain
0.00 Message "Load the sample loop with sample
    then press Continue", Screen
0.00 Hold
0.10 InjectionValve Inject
5.00 InjectionValve Load
 5.00  End_block
```

Pause suspends execution of the method and stops the pumps so that the system comes to a standstill. In ÄKTAdesign systems valves remain in the position they were in before the pause. The pause may be defined as indefinite or for a given number of minutes. This instruction is most useful for stopping the system in the event of an unexpected condition.

In both cases, the method may be resumed by pressing the **Continue** button in the System Control toolbar.

A third way for pausing a method, **Hold_until**, is described on page 113.

5.10.6 Linear flow rates

Linear flow rates (cm/h) can be specified for **Flow** instructions if a column is selected on the **Variables** tab or for the **Base** instruction in the block (see "Base instruction" on page 103). The volume flow rate is calculated from the specified linear flow rate and the column diameter as given in the column definition. To use a linear flow rate, check the **Linear Flow** option in the instruction dialogue for the **Flow** instruction. The calculated volumetric flow rate is shown during runs.

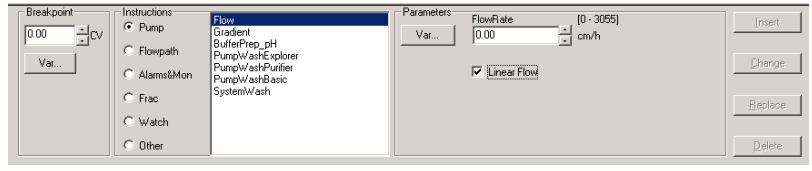


Fig 5-62. Setting linear flow rate in a method block. The linear flow rate option is only available if the base is set in terms of a named column definition.

Note: If a column is not selected in the **Base** instruction, linear flow will not be available.

5.10.7 Eluent concentrations and gradients

Both gradients and immediate changes in eluent concentration are defined in UNICORN methods with the single **Gradient** instruction.

The **Gradient** instruction takes two parameters:

Target which is the final eluent composition expressed in % eluent B

Length which is the duration of the gradient (not the breakpoint at the end of the gradient).

The instruction may be read as "form a **Gradient** to reach **Target** after **Length**". The starting point for the gradient is always the current eluent composition.

For example:

```
10.00 Gradient 50{%B}, 20{base}
```

forms a gradient to 50% B starting at breakpoint 10 with duration 20 method base units (i.e. finishing at breakpoint 30). If the current eluent concentration is greater than 50% B, the gradient will be negative.

Forming a step gradient

A step gradient is an immediate change in eluent composition. To form a step gradient in UNICORN, set the **Length** parameter to 0 in the **Gradient** instruction.

For example:

```
10.00 Gradient 50{%B}, 0{base}
```

forms a step from the current eluent composition to 50% B at breakpoint 10. The method continues with 50% B.

Setting the initial eluent composition

To set the initial eluent composition, set a step gradient to the required concentration at breakpoint 0 at the beginning of the method. This instruction can suitably be placed in the **Main** block, e.g. to start at 50% B:

```
(Main)
0.00 Base CV, (10)#Column_volume
0.00 Gradient 50{%B}, 0{base}
  ...
```

Breakpoints during gradients

The breakpoint for a **Gradient** instruction defines the time or volume (according to method base) for the start of the gradient. A gradient with a non-zero duration occupies time and volume in the method, and breakpoints for other instructions may be set to occur before the gradient is completed. For most instructions, the instruction is simply carried out at the requested breakpoint, while the gradient is forming. The following instructions affect the gradient, however:

- a new **Gradient** instruction will start a new gradient at the requested breakpoint. Any remaining duration of the previous gradient is ignored.
- a new **Flow** instruction will change the eluent flow rate at the requested breakpoint. If the current base is volume or column volume, the duration of the gradient will be changed. If the method base is time, the volume of the gradient will be changed.
- an **End_Method** instruction will stop the whole method, interrupting the gradient.

If an **End_Block** instruction is encountered during a gradient, the gradient formation will continue uninterrupted unless a new **Gradient** instruction is issued in the next block. This means for example that a block can be called conditionally during gradient formation without interrupting the gradient.

Gradients with variable duration

For many purposes, it can be useful to define the length of the gradient as a variable. When this is done, breakpoints for instructions issued during or after the gradient in the same block are automatically shifted in proportion to the length of the gradient, with the same functionality as **Change** in the text instruction editor (see "Changing instructions" on page 61).

Note: **Gradient** functions over time and any instruction that you want to insert after a gradient should be placed after the combined breakpoint and gradient length.

5.11 How to use selected conditional method instructions

Conditional instructions allow the progress of a run to be determined by the events during the run, e.g. start collecting fractions when the first peak elutes, or equilibrate the column until the eluent conductivity has reached a given value. This is facilitated by the **Watch** instructions.

5.11.1 Hold_until instruction

Hold_until is a special kind of **Watch** instruction. The method is put on hold until a specific condition is met (signal, test or value) or the timeout is reached. Thereafter the remaining instructions in the method are executed. Instructions that share the same breakpoint as the **Hold_until** instruction, but are placed after it in the method, will be executed after the **Hold_until** conditions have been met.

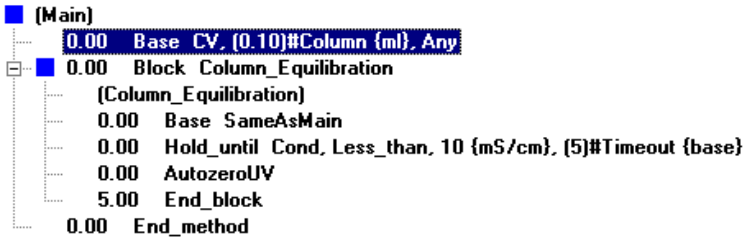


Fig 5-63. Example of a Hold_until instruction in a method.

5.11.2 Standard Watch conditions

The system strategy includes **Watch** instruction for each monitor defined in the system. A **Watch** is active from the point at which it is issued until either:

- the **Watch** condition is met
- a new watch is set for the same monitor
- a **Watch_Off** instruction is issued for the monitor

The conditions for which a **Watch** can be set are as follows for most monitors:

Greater_than	The signal exceeds a specified value.
Less_than	The signal falls below a specified value.
Slope_greater_than	The rate of change of the signal exceeds a specified value, expressed in monitor units/minute (e.g. mAU/min).
Slope_less_than	The rate of change of the signal falls below a specified value, expressed in monitor units/minute (e.g. mAU/min).
Less_than_or_valley	The signal falls below a specified value or a valley is detected. A valley is detected only

after a peak_maximum has been detected, and the valley is defined by a local minimum followed by an increase to 102% of the local minimum value plus the **Delta_Peak** value (see below).

Peak_max The signal falls to a specified fraction of the most recent peak maximum minus the **Delta_Peak** value (see below). **Factor**=1 detects peak maximum.

Stable_baseline The signal is stable within the limits of the **Delta_Base** value (see below) for the period specified by the minutes parameter.

To determine suitable values for watch conditions, it is often most convenient to examine data from a test run. For slope values, use the **Differentiate** function in the evaluation module to measure the slope of the test chromatogram (see "Finding the slope values for Peak Fractionation or Watch instructions" on page 287). The Simulate Peak Fractionation technique (see "Finding the slope values for Peak Fractionation or Watch instructions" on page 287) can also be used to find the slope values.

Two conditions apply for air sensors, although may be handled differently dependent on the system:

Equal 0 No air detected.

Equal 1 Air detected.

Note: To use the **Watch_AirSensor** instruction for air sensors, the **Alarm_AirSensor** setting in **Alarms&Mon** must be disabled (use the Method Editor to disable the alarm locally in a method, or the settings to disable the alarm for all methods). The **Alarm_AirSensor** setting overrides any **Watch_AirSensor** instruction, and if the alarm is enabled the method will pause when air is detected.

Settings for watch conditions

The mode of function of the **WatchPar** "Monitor" instruction in ÄKTA design systems is affected by certain parameters within the instruction (see below).

Delta_Peak
The **Delta_Peak** setting helps the software to detect valleys, peaks and Peak maximum, and to ignore noise in the chromatogram.

The **Delta_Peak** setting:

(a) sets the threshold for signal increase after a local minimum which will be interpreted as a valley for the **Less_than_or_valley** condition. A valley and a new peak are detected when the signal increases to 102% of the local minimum plus the **Delta_Peak** value. Note that a valley is detected only after a peak_maximum has been detected.

For example, if there is a local minimum at 0.05 AU and a **Delta_Peak** of 0.01 AU, a valley will be detected at:

(1.02 x 0.05) + 0.01 = 0.111 AU

(b) sets the threshold for signal decrease after a local maximum which will activate the **Peak_max** condition. **Peak_max** is detected when the signal falls to the specified fraction of the most recent peak maximum minus the **Delta_Peak** value.

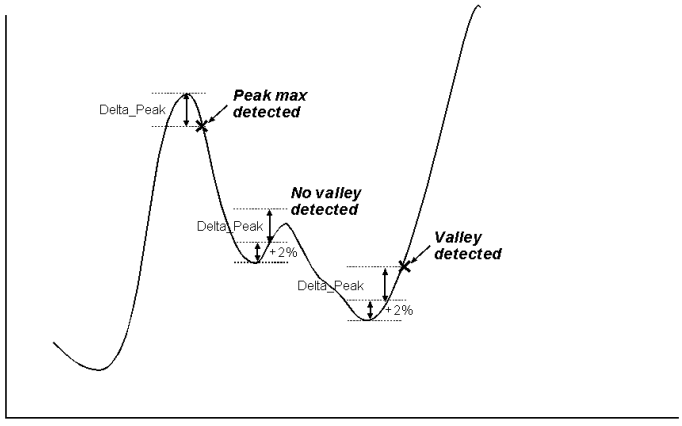


Fig 5-64. Illustration of the Delta_Peak setting. Peak max is detected when the signal falls by Delta_Peak from a local maximum if the Peak_max factor is set to 1.

The **Delta_Peak** value should be set large enough so that signal noise does not activate the conditions, and small enough so that the condition is activated close to the valley or peak. As a general guideline, set the value to 2-3 times the noise level and 5-10% of the smallest expected peak height. Setting a value that is too high can prevent a new peak from being detected after a local minimum.

Delta_Base

Delta_Base helps the software to determine when the baseline is considered to be stable, i.e. it defines the permitted variation for the

Stable_baseline condition. For this condition to be activated, the signal may not vary by more than the **Delta_Base** value up or down over the time interval specified in the **Stable_baseline** condition in the watch instruction.

The **Delta_Base** setting affects only the **Stable_baseline** condition.

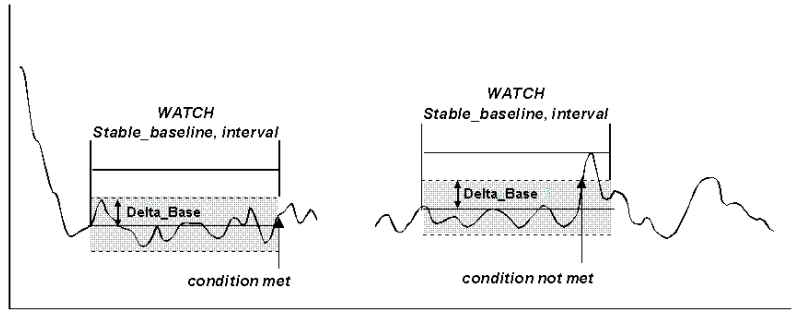


Fig 5-65. Illustration of the Delta_Base setting. The condition Watch Stable_baseline is met if the signal does not deviate by more than ±Delta_Base from baseline during the time interval specified for the watch. The baseline value is determined by the signal at the start of the watch. If the condition is not met, a new interval is started with a new baseline value defined by the signal level at the start of the new interval.

Permanent settings for **Delta_Peak** and **Delta_Base** are entered with the **WatchPar** instruction (e.g. **WatchPar_UV, WatchPar_Cond**) under **System:Settings** in the System Control menu (see the UNICORN 4.0 Administration and Technical manual). Temporary settings that apply only for the duration of a given run can be entered with the **WatchPar** in the **Alarms&Mon** group in the Method Editor.

Watch actions

The following actions can be taken when a watch condition is met:

Block name	Call the named block.
Pause, Hold	Pause or hold the method
Continue	Continue the method if paused or held
End_block	End the current block and return to the point from which the block was called
End_method	End the method

Ready	Indicates that the next step in a MethodQueue may start.

5.11.3 Watch examples

Below follows several examples of how **Watch** instructions may be inserted into an ordinary template method. Some of these methods are also on the UNICORN installation CD-ROM.

Watch example 1: Simple equilibration

In the following example:

The **Watch** is started on the conductivity signal and the method is then put on **Hold**. When the **Watch_cond** condition is fulfilled, **Continue** is issued and **Watch_cond** is turned off automatically. Method execution now continues issuing a **Watch_UV** command. Again the method is put into **Hold** until the **Watch** condition is fulfilled. Note that even though the line,

```
Watch_Cond, Less_than, 5 {mS/cm}, Continue
```

is in the method placed before the "**Hold**", the method is put on hold first and then continued only after the conductivity has reached a level less than 5 ms/cm. This is because "**Hold**" is an instruction, which will be executed at its breakpoint, while "**Continue**" is not an instruction but rather an action for the **Watch** instruction.

This method works satisfactorily although one drawback is that it might never end, and thus consume all of the buffer if the conditions for some reason are unfulfilled. See the **Watch** example 2 below for equilibration with a safeguard.

Note: If you are not using ÄKTA instruments a delay of 0.1 ml/min should be added after the **Hold**/**Pause** instruction so that the following instruction will not be executed simultaneously with the **Hold**/**Pause** instruction.

```
(Main)
0.00 Base CV, 0.96{ml}, Resource_Q_1_ml
...
0.00 Block EQUILIBRATE
  (Equilibrate)
  0.00 Base SameAsMain
  0.00 Watch_Cond, Less_than, 5 {mS/cm}, Continue
  0.00 Hold
```

```
0.10 Watch_UV, Less_than, 100 {mAU}, Continue
0.10 Hold
0.10 End_block
```

Watch example 2: Equilibration with simple safeguard
This is an example of equilibrating the column until the UV has reached a level below 100 mAU or until the column has been equilibrated with five column volumes of buffer, whichever condition is met first. In this way it is possible to equilibrate the column without the risk of running out of buffers.

```
(Main)
0.00 Base CV, 0.96{ml}, Resource_Q_1_ml
...
0.00 Block EQUILIBRATE
 (Equilibrate)
 0.00 base SameAsMain
 0.00 Watch_UV, Less_Than, 100 {mAU}
 End_block
 5.00 Watch_Off UV
 5.00 Message “The condition was never reached”
 5.00 End_block
```

Watch example 3: Equilibration with extra safeguard
The column is equilibrated until the conductivity is below 5 mS/cm. If this value is not reached within 6 column volumes, the method is paused together with a message. Equilibration of the column is continued until the conductivity value is "stable" (allowed to vary by max ±2 mS/cm) over a period of at least 5 minutes. If this condition is not met within 10 column volumes, the method is again paused. At each pause, the operator can decide whether to continue or abort the run.

Note: If you are not using ÄKTA instruments a delay of 0.1 ml/min should be added after the **Hold/Pause** instruction so that the following instruction will not be executed simultaneously with the **Hold/Pause** instruction.

```
(Main)
0.00 Base CV, 0.96 {ml} Resource_Q_1_ml
```

```
0.00 Block EQUILIBRATE
    (Equilibrate)
    0.00 Base SameAsMain
    0.00 Block COND_LESS_THAN
    0.00 Block COND_STABLE
    0.00 End_block
    (Cond_less_than)
    0.00 Base SameAsMain
    0.00 Watch_Cond, Less_than, 5 {mS/cm},
    End_block
    6.00 Message "Low conductivity not reached",
    Screen
    6.00 Pause -1.00 {minutes}
    6.00 End_block
    (Cond_stable)
    0.00 Base SameAsMain
    0.00 WatchPar_Cond, 0.50 {mS/cm}, 2 {mS/cm}
    0.00 Watch_Cond, Stable_baseline, 5
    {minutes}, End_block
    10.00 Message "Conductivity not stable",
    Screen
    10.00 Pause -1.00 {minutes}
    10.00 End_block
```

Watch example 4: Safe sample injection

The example below applies to systems that have air sensors. Inject a 50 ml sample with the system pump directly on to the column. If there is less than 50 ml of sample so that air is detected in the inlet, the block will be ended and the method continued. The **Watch_AirSensor1** instruction sets a watch on the inlet air sensor.

```
(Main)
0.00 Base CV (0.10)#Column {ml} Any
  ...
  0.00 Block Load_Sample_with_SystemPump
  (Load_Sample_with_SystemPump)
```

```
0.00 Base Volume
0.00 Alarm_AirSensor1 Disabled
0.00 BufferValveA1 (A18)#Sample_inlet
0.00 Gradient 0.00 {%B} 0.00 {base}
0.00 InjectionMark
0.00 Flow (1.00)#Injection_Flow_rate {ml/min}
0.00 Watch_AirSensor1 Equal, 1, END_BLOCK
(50.00)#Volume_of_sample End_block
0.00 Block Complete_Sample_Loading_Sys
(Complete_Sample_Loading_Sys)
0.00 Base Volume
0.00 Watch_off AirSensor1
0.00 Block Initial Eluent Conditions_Sys
    (Initial Eluent Conditions_Sys)
    0.00 Base Time
    0.00 PumpAInlet A1
    0.00 BufferValveA1 (A11)#Eluent_A_inlet
    0.00 PumpBInlet (B1)#Eluent_B_inlet
    0.01 Gradient 0.00 {%B} 0.00 {base}
    0.01 Flow (1.00)#Flow_rate {ml/min}
    0.11 End_block
20.00 End_block
...
```

Watch example 5: Collecting three absorbance peaks

Collecting three absorbance peaks through outlets F3, F5 and F7 with Waste fractions through outlets F4, F6 and F8, is described below.

Note 1: The waste fractions between the peaks are collected through the outlet valve positions F4, F6 and F8, so ensure that the tubing from these positions is lead to a suitably large container.

Note 2: It is not recommended to use outlet fractionation for peaks with small volumes, i.e. less than 10 ml. The fraction collector should be used for such peaks.

The maximum number of peaks collected in this example is three due to the limited number of positions on the outlet valve. When the UV reaches 5 mAU or more the outlet valve is switched to the position for collecting the first peak. When the UV reading goes down to 4.75 mAU the outlet valve switches to the position for collecting the first peak fraction. This process is repeated twice for the next two peaks so that when the UV reading rises above 5 mAU the position switches to collect the peak fraction and the position switches again to collect the waste fraction when the UV reading falls again.

Note: The UV threshold for collecting the waste fraction must be below the threshold for collecting the peak fraction so that the "waste" condition will not be fulfilled simultaneously or immediately after peak collection.

```
(Main)
 0.00 Base CV (0.10)#Column {ml} Any
 ...
 0.00 Block Gradient
 (Gradient)
     0.00 Base SameAsMain
     0.00 Gradient (100)#Target_ConcB {%B}
     (20)#Length_of_gradient {base}
 0.00 Block Eluate_Fractionation
     (Eluate_Fractionation)
     0.00 Base SameAsMain
     0.00 FractionationStop
     0.00 OutletValve WasteF1
     0.00 Watch_UV Greater_than, 5 {mAU}, Peak
         (Peak)
         0.00 Base SameAsMain
         0.00 OutletValve Feed
         0.00 Watch_UV Less_than_or_valley, 4.75
     {mAU}, Waste
             (Waste)
             0.00 Base SameAsMain
             0.00 OutletValve Feed
```

```
        0.00 Watch_UV Greater_than, 5 {mAU},
Peak1
            (Peak1)
            0.00 Base SameAsMain
            0.00 OutletValve Feed
            0.00 Watch_UV Less_than_or_valley,
4.75 {mAU}, waste1
                (waste1)
                0.00 Base SameAsMain
                0.00 OutletValve Feed
                0.00 Watch_UV Greater_than, 5
{mAU}, Peak2
                    (Peak2)
                    0.00 Base SameAsMain
                    0.00 OutletValve Feed
                    0.00 Watch_UV
Less_than_or_valley, 4.75 {mAU}, END_BLOCK
                    0.00 End_block
                0.00 End_block
            0.00 End_block
        0.00 End_block
    0.00 End_block
0.00 OutletValve WasteF1
0.00 End_block
20.00 End_block
```

Watch example 6: Collecting absorbance peaks

Collect one or two absorbance peaks through outlets F3 and F4 respectively with waste fractions collected through outlet valve F1 (waste). Each called block (except **End_collect**) resets the Watch condition so that the method will react correctly to subsequent changes in the UV absorbance.

```
(Main)
0.00 Base CV, Resource_Q_1_ml
```

```
...
0.00 Block ELUTION
(Elution)
0.00 Base SameAsMain
0.00 Gradient 100 {%}, 20 {Base}
0.00 Watch_UV, Greater_than, 100 {mAU}, Peak_1
20.00 Message "End of gradient", Noscreen
20.00 End_block
(Peak_1)
0.00 Base SameAsMain
0.00 OutletValve F3
0.00 Watch_UV1, Less_than_or_valley, 100 {mAU},
    Waste
0.00 End_block
(Waste)
0.00 Base SameAsMain
0.00 OutletValve F1
0.00 Watch_UV1, Greater_than, 100 {mAU}, Peak_2
0.00 End_block
(Peak_2)
0.00 Base SameAsMain
0.00 OutletValve F4
0.00 Watch_UV1, Less_than, 100 {mAU}, End_collect
0.00 End_block
(End_collect)
0.00 Base SameAsMain
0.00 OutletValve F1
0.00 End_block
```

• p123

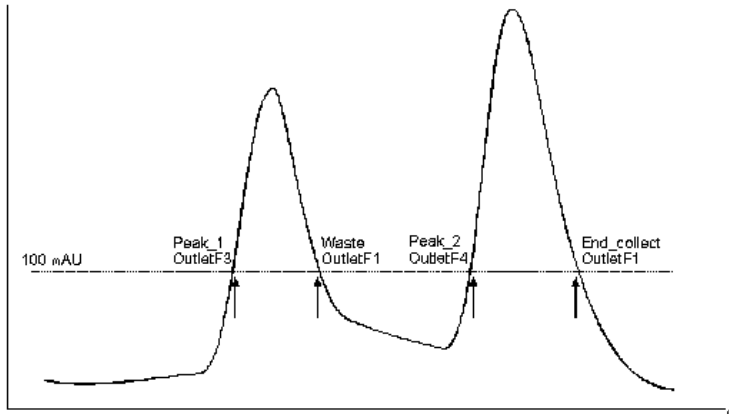


Fig 5-66. Peaks collected by the method in example 4 above.

The design of a method of this kind (with several **Watch** instructions for the same monitor) is important. The following construction appears simpler but is incorrect:

Wrong!

```
...
0.00 Watch_UV, Greater_than, 100 {mAU}, Peak_2
0.00 Watch_UV, Less_than, 100 {mAU}, End_collect
0.00 End_block
(Peak_2)
0.00 Base SameAsMain
0.00 OutletValve F4
0.00 End_block
(End_collect)
0.00 Base SameAsMain
0.00 OutletValve F1
 0.00 End_block
```

Here, the second **Watch** instruction will annul the first since a signal can only be watched for one condition at a time. In the correct construction above, the watch to end collection is not set until **Peak_2** is called.

Watch example 7: Collecting peaks above a threshold value

The example below describes how to use the **Watch** instruction to collect three peaks above a certain threshold value such as UV, instead of using slope for the **Peak_Fractionation** instruction. The number of peaks to be collected can be increased by adding several **Start_Threshold** and **Ending_Threshold** blocks. When the UV reading goes up to 5 mAU and beyond, the fraction collector is started. The desired fraction volume must be entered for the **FractionCollector** instruction. When the UV reading goes down to 4.75 mAU the fractionation is ended and the flow directed to Waste. This is repeated for the next two peaks.

Note: The UV threshold for ending fraction collection must be below the threshold for starting fraction collection so that end fractionation conditions will not be fulfilled simultaneously or immediately after start fractionation.

```
(Main)
0.00 Base CV 0.98 {ml} Mono_S_HR_5/5
...
0.00 Block Gradient
  (Gradient)
  0.00 Base SameAsMain
  0.00 Gradient (100)#Target_ConcB {%B}
      (20)#Length_of_gradient {base}
  0.00 Block Eluate_Fractionation
      (Eluate_Fractionation)
      0.00 Base Volume
      0.00 OutletValve WasteF1
      0.00 Watch_UV Greater_than,
      (5)#Start_Threshold {mAU),
      Start_Fractionation
          (Start_Fractionation)
          0.00 Base SameAsMain
          0.00 FractionCollector
      (1)#Eluate_FracSize {ml}
          0.00 Watch_UV Less_than_or_valley,
      (4.75)#Ending_Threshold {mAU},
      End_Fractionation
              (End_Fractionation)
```

```
        0.00 Base Volume
        0.00 FeedTube
        0.00 FractionationStop
        0.10 Watch_UV Greater_than,
(5)#Start_Threshold1 {mAU},
Start_fractionation1
            (Start_fractionation1)
            0.00 Base SameAsMain
            0.00 FractionCollector
(1)#Eluate_FracSize1 {ml}
            0.00 Watch_UV Less_than_or_
valley, (4.75)#Ending_Threshold1 {mAU},
End_fractionation1
                (End_fractionation1)
                0.00 Base SameAsMain
                0.00 FeedTube
                0.00 FractionationStop
                0.10 Watch_UV Greater_than,
(5)#Start_Threshold2 {mAU},
Start_fractionation2
                    (Start_ fractionation2)
                    0.00 Base SameAsMain
                    0.00 FractionCollector
(1)#Eluate_FracSize2 {ml}
                    0.00 Watch_UV Less_than_
or_valley, (4.75)#Ending_threshold2 {mAU},
waste2
                        (waste2)
                        0.00 Base SameAsMain
                        0.00 FractionationStop
                        0.00 End_block
                    0.00 End_block
                0.10 End_block
            0.00 End_block
        0.10 End_block
```

```
        0.00 End_block
    20.00 End_block
  ...
0.00 End_method
```

Watch example 8: Starting and stopping fractionation at a certain concentration of Buffer B

Sometimes you may wish to start and stop fractionation at a certain concentration of Buffer B. The example below shows you how to do this with the **Watch** instruction. Set the desired start fractionating concentration to the first **Watch_Conc** instruction, and insert the desired slope and peak fractionation size to the **Peak_Frac_Parameters** and **Peak_Fractionation** instructions. The peak fractionation will then start at the desired concentration. Set also the desired end concentration of buffer B to the second **Watch_Conc** instruction to end the peak fractionation.

```
(Main)
0.00 Base CV (0.10)#Column {ml} Any
  ...
  0.00 Block Gradient
      (Gradient)
      0.00 Base SameAsMain
      0.00 Gradient (100)#Target_concB {%B}
      (29)#Length_of_gradient {base}
      0.00 Block eluate_and_Peak_Fractionation
          (Eluate_and_Peak_Fractionation)
          0.00 Base SameAsMain
          0.00 FractionationStop
          0.00 OutletValve WasteF1
          0.00 FractionCollector (0.00)#fracsize
      {ml}
          0.00 Watch_Conc Greater_than,
      (0)#Start_Peak_Frac_at {%B},
      Start_Peak_Fractionation
              (Start_Peak_Fractionation)
              0.00 Base Time
              0.00 Peak_FracParameters
```

```
(100.00)#Peak_Start_Slope {mAU/min},
(75.00)#Peak_End_slope {mAU/min},
(0.15)#Minimum_peak_width {min}

       0.00 Peak_Fractionation
(0.5)#Peak_FracSize {ml}

       0.00 Watch_Conc Greater_than,
(100)#End_Peak_Frac_at {%B},
End_Peak_Fractionation

           (End_Peak_Fractionation)

           0.00 Base Time

           0.00 Peak_FracStop

           0.00 End_block

       0.00 End_block

   0.00 End_block

20.00 End_block

...

0.00 End_method
```

5.12 The Column list

It is possible to select a column in combination with a template when creating a new method (see "Creating a new method" on page 33). Certain column-specific parameters are automatically copied into the method. Available columns are found under parameter **Column** in the **New Method** box and new columns can be added to this list and then be selected when a method is created. The Column list is not linked to a particular method, although the columns are edited within the Method Editor. Columns are either globally available, i.e. available to all users, or personal, i.e. not available to other users. It is best not to edit the globally available columns unless you save the changes under a new column name, since other users may not appreciate the changes. It is recommended that restricted access be given to editing global columns (see the UNICORN 4.0 Administration and Technical manual for details on restricting access).

5.12.1 Adding a new column

To add a new column to the Column list, do the following:

1. Select **Edit:Column List** to open the **Column List** dialogue. Selection of a column in the list will display its parameters in the field to the right of the box.

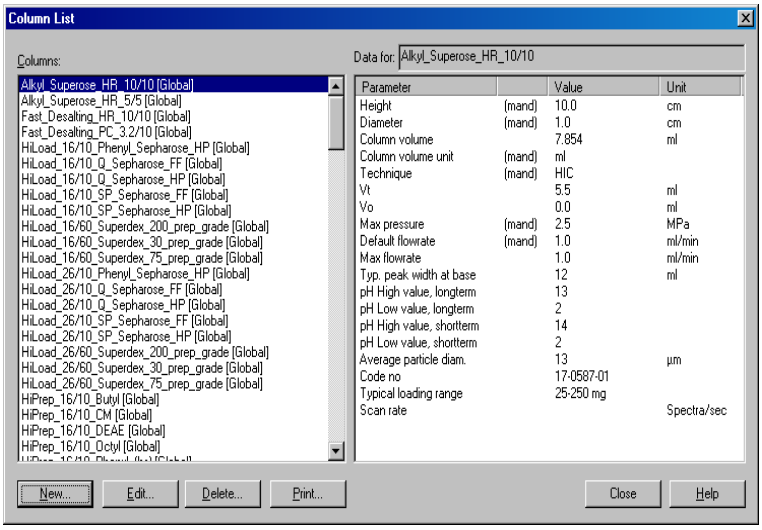


Fig 5-67. The Column List dialogue.

2. Click on **New** to display the **New Column** dialogue.

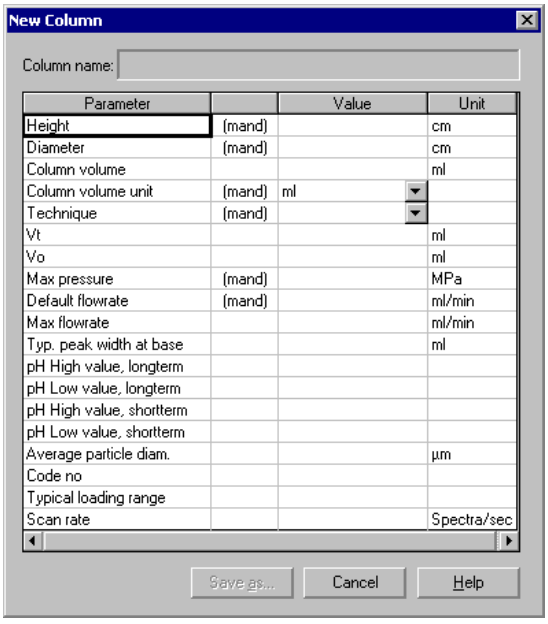

New Column
Column name:
Parameter | | Value | Unit
Height | (mand) | | cm
Diameter | (mand) | | cm
Column volume | | | ml
Column volume unit | (mand) | ml | |
Technique | (mand) | | |
Vt | | | ml
Vo | | | ml
Max pressure | (mand) | | MPa
Default flowrate | (mand) | | ml/min
Max flowrate | | | ml/min
Typ. peak width at base | | | ml
pH High value, longterm
pH Low value, longterm
pH High value, shortterm
pH Low value, shortterm
Average particle diam. | | | µm
Code no
Typical loading range
Scan rate | | | Spectra/sec
Save as...
Cancel
Help

Fig 5-68. The New Column dialogue.

3. The **Parameter** column lists the column parameters that can be altered. Some of the parameters are mandatory (labelled **mand**) and you cannot save the column until these have been filled in. Use the **Variables** column to enter the desired values.

   The column parameters are used in UNICORN in the following ways:

Parameter	*Unit*	*Mandatory*	*Comments*
Height	cm	yes	Calculation of N/m. Calculation of column volume
Diameter	cm	yes	Calculation of volumetric flow, from a set linear flow. Calculation of column volume
Column volume	ml or µL or nl or litre	yes	Used in method with CV as base. Automatically calculated from **Height** and **Diameter**. Not possible for user to set directly
Column volume unit	ml or µl or nl or litre	no	The column volume is calculated in the set unit. The set unit is also used for Vt, Vo and flow rates
Technique		yes	Decides for which technique the column should be available when **New:Method** is selected
Vt	ml or µl or nl or litre	no	Total liquid volume, used to calculate the capacity factor after an integration
Vo	ml or µl or nl or litre	no	Void volume, used to calculate K_{av} after integration

Max pressure	MPa	yes	Used for setting pressure limit automatically in method. Warning when method is saved and started if the pressure limit is set to higher value
Default flowrate	ml/ min or litre/h or µl/min or nl/min	yes	Used to set the flow rate automatically in a method. If the method uses linear flow, the parameter value is automatically calculated
Max flowrate	ml/ min or litre/h or µl/min or nl/min	no	Used to give a warning when saving or starting a method if a higher flow rate is chosen
Typ. peak width at base	ml or litre or µl or nl	no	Used to set averaging time for UV detector. Used to set peak fractionation parameters
pH High value, long-term		no	Used to give a warning when saving or starting the method if the **BufferPrep_pH** is higher than the set value
pH Low value, long-term		no	Used to give a warning when saving or starting the method if the **BufferPrep_pH** is lower than the set value
pH High value, short-term		no	Used to give a warning when saving or starting the method if the **BufferPrep_pH** is higher than the set value

pH Low value, short-term		no	Used to give a warning when saving or starting the method if the **BufferPrep_pH** is lower than the set value
Average particle diameter	µm	no	Information only
Code No		no	Information only
Typical loading range	mg	no	Information only
Scan rate	spectra/sec	no	Information only

Note 1: The values for the parameters **Max pressure**, **Default flowrate** and **Typical peak width at base** (used to set average time and peak fractionation parameter **MinWidth**) are only copied into the method if the corresponding instructions are available as variables.

4. Click on **Save as** and enter the name of your column. You can choose to save the column globally, i.e. available to all users, by checking the **Save as global** box. If the column is instead for your personal use only, uncheck the **Save as global** box. A personal and global column cannot be given the same name. Click on **OK** to add your column to the column list.

Note: Saving column globally requires **Edit global lists** authorisation in the user profile (see the UNICORN 4.0 Administration and Technical manual).

5.12.2 Editing column parameters

Column parameters may be edited by doing the following:

1. Select **Edit:Column List**. Select a column and then click on the **Edit** button to display the **Edit column** dialogue.

2. Use the Values column to alter any desired parameter settings.

3. To save the changes in place of the original parameters, click on **Save**. To save the changed parameters as a new column, click on **Save as**. In the **Save As** dialogue you can change the name of the column. Select (check) the **Save as global** box if you want the column to be globally available. If the column is instead for your personal use only, deselect (uncheck) the **Save as global** box. Click

on the **OK** button and the new column will be added to the column list.

Note: If parameters for a certain column are changed and the same column was earlier selected and saved in a method, the column in the method will not automatically be updated with the new parameters. When opening the method, a question will appear asking if you want to update the parameters. It is recommended that you answer **Yes**.

5.12.3 Deleting a column

To delete a column from the list, mark the column and click on the **Delete** button. Be restrictive in deleting global columns since these are available to all users.

5.12.4 Selecting or changing the column in a method

Any column may be selected together with the appropriate template in the **New Method** dialogue (see "Creating a new method" on page 33). The column can later be changed in the **Instruction** box.

It is also possible to adapt a method to different columns if the column definition name is defined as a variable in the **Base** instruction (as it is in the method templates or Wizard-generated methods). Changing the column for a method is then done in the Variables tab.

5.13 Creating and editing BufferPrep recipes

The BufferPrep function is available on some ÄKTAdesign systems. Recipes can be edited or new ones created by selecting **Edit:BufferPrep Recipes** in the Method Editor. The list of recipes is not linked to a specific method, although editing is performed in the Method Editor. Recipes are either global, i.e. available to all users, or personal, i.e. not available to other users. It is best not to edit the global available recipes unless you save the changes under a new recipe name, since other users may not appreciate the changes. It is recommended that restricted access be given to editing global recipes.

Note: The recipe to use in a certain method is selected in the **BufferPrep** tab in the Run Set-up (see "BufferPrep" on page 88).

5.13.1 Creating a BufferPrep recipe

To create your own recipe do the following:

1. Open the **BufferPrep Recipes** dialogue by selecting **Edit:BufferPrep Recipes** in the Method Editor.

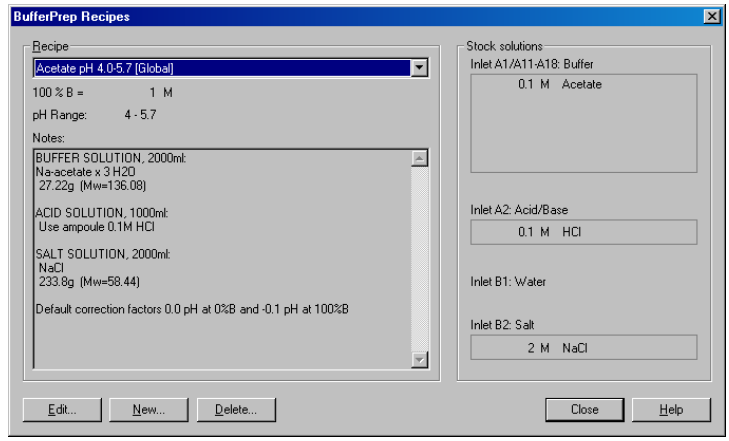


Fig 5-69. BufferPrep Recipes dialogue.

2. Click on **New** to open the **New Recipe** dialogue and select a buffer from the **Buffer substances** drop-down list. If the required buffer is not listed, see Defining a new buffer substance (below).

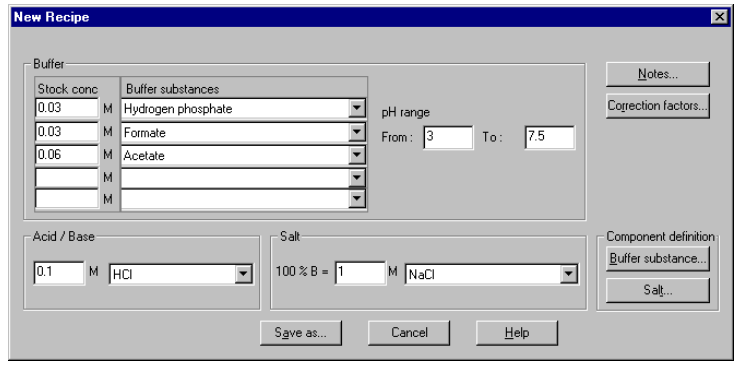


Fig 5-70. The New Recipe dialogue.

Insert the intended stock concentration for the selected buffer. Use a buffer concentration of 2-4 times higher than the concentration used in the normal preparation. When **BufferPrep** is used, the buffer will be diluted between 2-10 times dependent on the amount of acid/base that has to be used to reach the desired pH.

Up to five buffering components can be selected in the **Buffer** field. To prevent too high ionic strength, the sum of the concentrations for all buffers selected should be between 0.03 M and 0.2 M (typically 0.1 M).

3. Select either **HCl** (acid) or **NaOH** (base) from the **Acid/Base** pull-down list and insert the required stock concentration (typically 0.1 M).

4. Select a salt from the pull-down list. To create new salts; see Defining a new salt (below). Set the maximum outlet concentration of the salt for 100%B (usually 1.0 M).

5. Enter the desired pH range of the buffer.

Note: To set a useful pH range the pKa must be known. Click on the **Buffer substance** button and select the buffer component. The pKa values are shown in the list. Typically, a pH range ±0.5 units around the pKa is useful. For exact ranges check buffer tables.

6. To add notes about the recipe, click on **Notes** and enter the required text in the displayed box. Click on **OK**.

7. When you have created your recipe, click on **Save as**. If you have entered values into your recipe that are not feasible, a warning message will be displayed that tells you what is wrong.

   Enter a name and save the recipe either as global or personal by checking/unchecking the **Save as global** box. Click on **OK** to add the recipe to the **BufferPrep** list.

Defining a new buffer substance

Before defining a new buffer substance ensure that all pKa values for the substance are available. The pKa values entered should be true pKa (i.e. the pKa value at indefinite dilution) as opposed to apparent pKa (i.e. measured at a non-zero concentration). The pKa values should be given at 25° C.

1. In the **New Recipe** dialogue, click on the **Buffer substance** button. Click on **New** button in the **Define buffer substance** box that appears and enter the name of the new component. Click on **OK**.

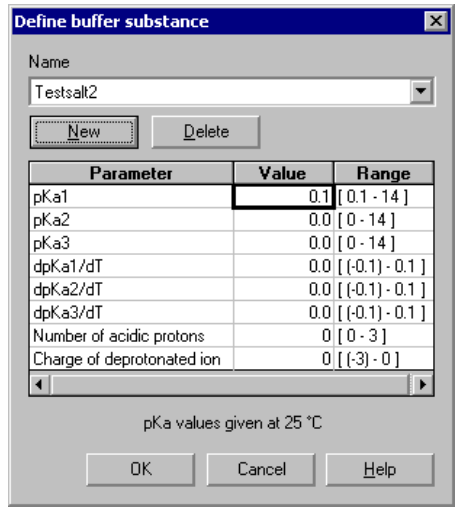

Define buffer substance
Name
Testsalt2
New
Delete
Parameter
Value
Range
pKa1 0.1 [0.1 - 14]
pKa2 0.0 [0 - 14]
pKa3 0.0 [0 - 14]
dpKa1/dT 0.0 [(-0.1) - 0.1]
dpKa2/dT 0.0 [(-0.1) - 0.1]
dpKa3/dT 0.0 [(-0.1) - 0.1]
Number of acidic protons 0 [0 - 3]
Charge of deprotonated ion 0 [(-3) - 0]
pKa values given at 25 °C
OK
Cancel
Help

Fig 5-71. The Define buffer substance dialogue.

2. Enter the appropriate values in the **Value** field for each pKa and dpKa/dT parameter. All values must fall within the stated **Range** limits. Up to three values can be entered for each buffering component. When the buffering component has less than three pKa's, the other values should be set to zero. A pKa value of zero means that the pKa does not change with temperature.

3. Enter the number of acidic protons for the buffer substance in the form that it is actually weighed in, e.g. for NaH_2PO_4 enter 2, for Na_2HPO_4 enter 1, for Tris enter 0.

4. Enter the charge of the completely de-protonated ion. This will be a negative value for an acid and zero for a base, e.g. for NaH_2PO_4 enter -3, for Tris enter 0.

5. Click on **OK** to add the new buffer substance to the list of available buffers.

Defining a new salt

When defining a new salt ensure that the new salt is inert, i.e. a salt with no buffering properties.

1. In the **New Recipe** dialogue, click on the **Salt** button. Click on the **New** button in the **Define salt** dialogue that appears and enter the name of the new component. Click on **OK**.

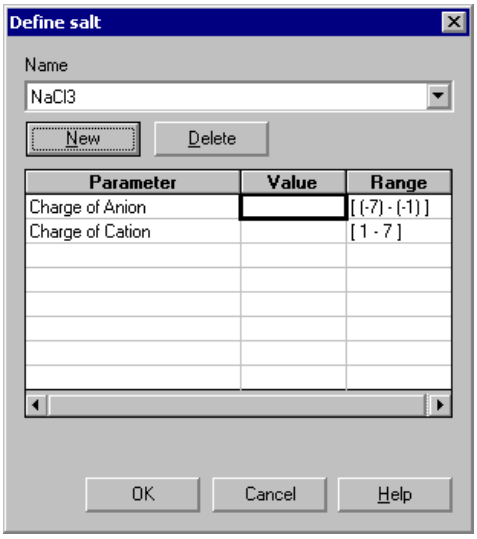


Fig 5-72. The Define salt dialogue.

2. Enter the charge of the anion, e.g. for Cl^- enter -1, for SO_4^{2-} enter -2.

3. Enter the charge of the cation, e.g. for Na^+ enter 1, for Mg^{2+} enter 2.

4. Click on **OK** to add the new salt to the list of available salts.

5.13.2 Editing a BufferPrep recipe

It is possible to edit an existing recipe or a recipe that you have created.

1. Select **Edit:BufferPrep Recipes** in the Method Editor. The **BufferPrep Recipes** dialogue will be displayed.

2. Select a recipe from the pull-down list and all information about the recipe will be displayed.

3. Click on the **Edit** button to display the recipe parameters.

   Change the recipe (see "BufferPrep" on page 88 for a description of the functions and see below for a description of correction factors) and save using either **Save** or **Save as**. The original recipe will now be updated or a new recipe added to the list, respectively.

Note: If parameters for a certain recipe are changed and the same recipe was earlier selected and saved in a method, the recipe in the method will not automatically be updated with new parameters. When opening the method, a question will appear asking if you want to update the parameters in the method recipe. It is recommended that you answer **Yes**. The question will not appear if you only change the correction factors. The correction factors in the method recipe will not then be updated.

Fine tuning with correction factors

In order to obtain high pH accuracy a recipe can be fine tuned around a specific pH by setting correction factors. Run **BufferPrep** manually at 0% and 100% and change the default correction factors as follows:

1. Select the **Recipe** under group **Other** in **System Control:Manual**. Click on **Execute**. Set the pH in the instruction BufferPrep_pH in group **Pump**. Click on **Execute**. Set the flow rate to be used during the run in instruction **Flow**. Click on **Execute**.

2. Check the pH reading when stable. Allow at least 30 ml of eluent to pass through before expecting a steady pH reading.

3. Change to 100% B by setting the Gradient instruction in **Manual:Pump** to 100% for **Target** and 0 for **Length**. Click on **Execute**.

4. Check the pH reading when stable at 100% B.

5. If the readings are acceptable at both 0% and 100%, the correction factors do not need to be changed.

6. If the readings are not acceptable, click on **Correction factors** in the **Edit Recipe** dialogue (**File:BufferPrep recipes:Edit** in the Method Editor).

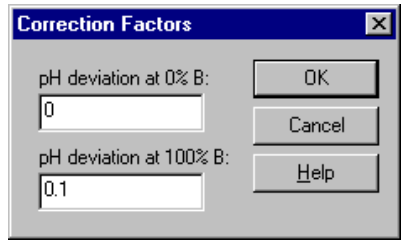


Fig 5-73. The Correction Factors dialogue.

7. Enter the deviation at 0% and 100%. (Example: if the pH is set to 7.0 and the actual pH is 7.1 enter 0.1, if the actual pH is 6.9 enter -0.1).

Note: If correction factors already exist, the measured pH deviation should be added to the old factors.

8. Click on **OK** and then save the recipe.

6 Performing a run

This chapter describes how to perform and monitor runs from the System Control module. Calibration of system monitors is also described.

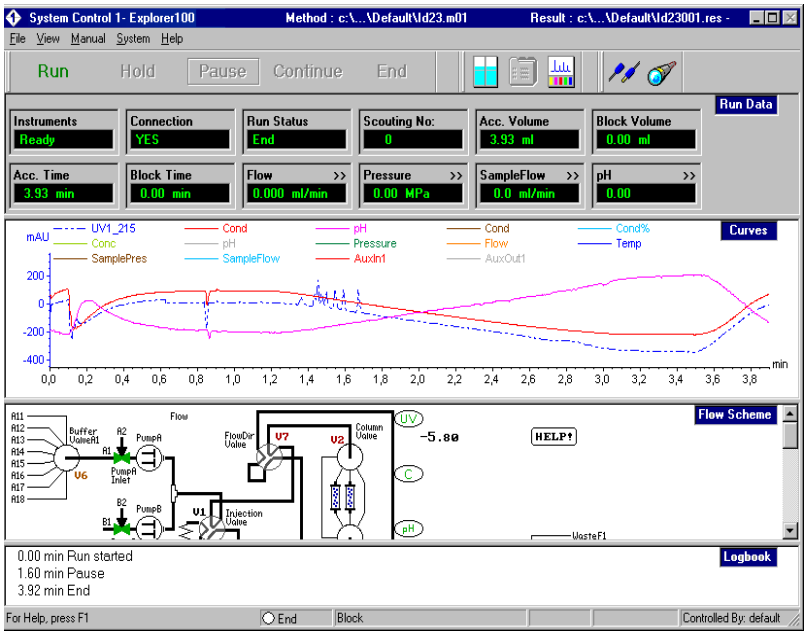


Fig 6-1. The System Control workspace with Run Data, curves, flow scheme and logbook displayed.

6.1 Starting a method

Methods can be started when the system is connected and no other method is running. You must have **Run methods** authorisation to start a method.

Before starting a method, make sure that:

- The correct system is connected in control mode.
- The system monitors are correctly calibrated

6.1.1 Starting from the Main Menu

You start a method from the Main Menu by highlighting a method in the **Methods** window and selecting **File:Run**. Alternatively, highlight the method and select **Run** from the shortcut menu.

Note: Double clicking the method opens the Method Editor with the method loaded.

6.1.2 Starting from System Control

From System Control select **File:Run** or click on the **Run** button on the toolbar, then double-click on the method icon in the displayed dialogue.

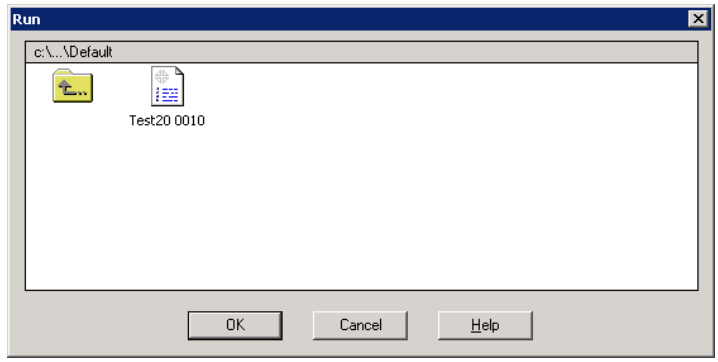


Fig 6-2. Starting a method from the System Control module.

For methods which are used frequently (e.g. column cleaning methods or routine separations), it may be convenient to define the methods as commands in the **File** menu. To do this, choose **File:Menu** and select the required method. The method name will appear as a command in the **File** menu, and choosing the command will start the method.

6.1.3 Starting an Instant Run

You can start a method template or wizard directly by clicking on the **Instant Run** icon in the Main Menu toolbar or by selecting **File:Instant Run** in System Control.

Note: Use of this function requires that templates or wizards are defined. Standard systems are supplied with templates or wizards. Custom systems require that the user creates templates.

6.1.4 Start protocol

If the method is defined with a start protocol this will be displayed before the method actually starts. Work through the start protocol, answering questions as required. As each screen is completed, click on **Next** to move to the next screen or **Back** to return to the previous screen. The last screen has a **Start** button to start the run. At any stage, click on **Cancel** to abort the method start.

The following start protocol items may be displayed (see "Run Set-up" on page 70):

Variables — All the variables defined in the method instructions, organised by block. Values for variables which are not defined as scouting variables can be changed here for the current run.

Scouting — Variables for scouting (see Chapter 7). Values for **Scouting** variables may be changed for the current run and override the corresponding values on the **Variables** tab. It is also possible to include or exclude runs in the scouting scheme with the **Run/ Excluded** buttons.

Text Method — Text instructions for the method. These are displayed for information only and cannot be changed at this stage.

Questions — Questions are data entry fields which are filled in by the operator when the run is started. Some questions may be mandatory and some may require authorised confirmation.

Notes — Method notes are shown and start notes can be entered.

Gradient — Displays the gradient for the method.

BufferPrep — Displays the recipe selected in the method. The correction factors can be changed for the current run. It is not possible to change recipe at the start of a run.

Columns — Displays columns selected in the method. Select a column and click on **View** to see the details of the column definition.

Reference Curve — Reference curves which may be displayed in the System Control workspace during the run can be selected here (see "Reference curves" on page 86).

Evaluation Procedures Evaluation procedures which will be executed automatically after completion of a run can be selected here (see "Evaluation procedures" on page 83).

Method Information Information about the method being run.

Settings Displays the system settings for the run. If the settings are not suitable, cancel the method start, change the settings with the **System:Settings** command (see the UNICORN 4.0 Administration and Technical manual) and restart the method.

Calibration Displays calibration data for system monitors. If the calibration is not acceptable, cancel the method start, re-calibrate the monitor(s) with the **System:Calibrate** command (see the UNICORN 4.0 Administration and Technical manual) and restart the method.

Result Name Changeable The names of the result file and scouting folder are specified here. This page is displayed if there are any other pages in the start protocol. The names may be changed if this is permitted in the start protocol.

Frac-950 Displays the set up for the Fraction collector. Also allows the user to set the last tube.

If there are any questions in the start protocol that require authorised confirmation, you will be asked for a username and password when you attempt to leave the screen containing the questions. Only users with **Confirm/Sign** authorisation may authorise answers to such questions. Each question that requires an authorisation must have a separate authorisation.

Note: If the start protocol for a method in the queue is cancelled, the MethodQueue is paused. Select **MethodQueue:Display Running** in the Main Menu and Restart or end the run in the displayed dialogue.

6.2 Monitoring a run

The System Control workspace displays the status of the current system. On the Windows taskbar, there may be up to four System Control modules available that can be connected to different systems. Separate systems may be controlled and displayed independently of each other.

Each System Control workspace displays up to four windows for monitoring different aspects of the run. Click on the **View Windows** icon or choose **View:Windows** from the menu to select which windows to display.

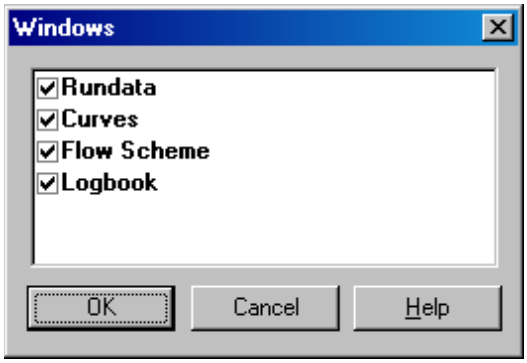


Fig 6-3. Dialogue for choosing windows to display in the System Control workspace.

6.2.1 General window techniques

Windows in the System Control are always displayed over the full width of the workspace. The split-bar between the displayed windows can be moved by selecting and dragging up or down to change the size of a specific window.

Any window can be maximized to the full view or restored to its original size by selecting the **Maximize** or **Restore** toggle command respectively in the shortcut menu.

To hide a window from view, select **Hide** from the shortcut menu.

6.2.2 Run data

The **Run Data** window displays the current values for selected run parameters. Values are updated at least every 5 seconds (the actual interval is defined in the system strategy).

Fig 6-4. The Run Data window.

Run data layout

The general display for the Run Data window can be selected using layouts by doing the following:

1. Select **View:Properties** or select **Properties** from the right click pop-up menu. The **Properties** dialogue is displayed for all windows in System Control.

2. Select the **Run Data Groups** tab.

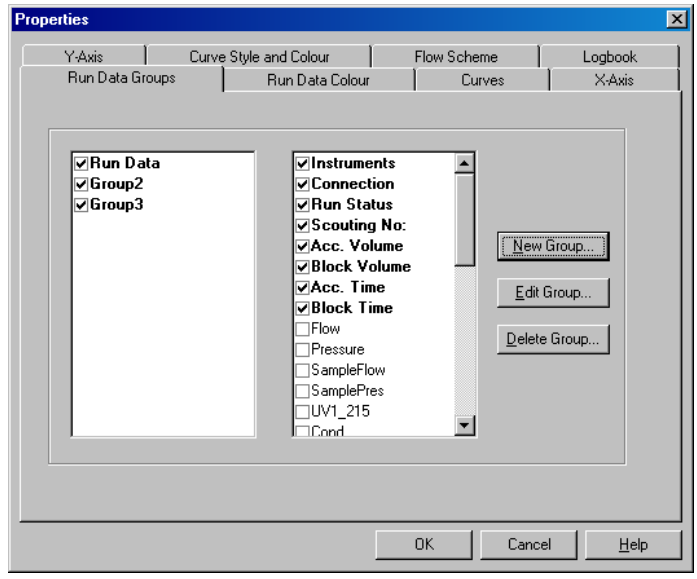


Fig 6-5. Properties dialogue, Run Data Groups tab.

3. Select individual run data parameters to view and click on **OK**.

Alternatively, select a group, either:

an available group	Single or multiple selections of available groups can be made by clicking the check boxes in the list to the left.
edit an available group	Select the group from the list on the left and click on the **Edit Group** button. Modify the included readings in the list to the right. Finish the dialogue by clicking on **OK**.
create a new group	Click on the **New group** button and select the readings that you want to view from the list, then enter a name for the group. Finish the dialogue by clicking on **OK**.

Note: For systems with optional components, parameters are not shown for components that are not included in the system.

4. To delete a group, click on **Delete Group** and click on the check boxes in the delete layout dialogue.

5. Click on **OK** to view the selection in the Run Data window. The name of the layout selected replaces the default layout name **Run Data**.

You can choose run data items to display without using named groups, simply be checking or unchecking items in the list.

Run data style

To change the colour of the text or text background in the displayed readings boxes:

1. With the cursor over the **Run Data** window, select **Colour Settings:Text** or **Colour Settings:Background** from the shortcut menu.

2. Select colours in the dialogue and click on **OK**.

Alternatively:

1. Select **Properties** from the shortcut menu. The **Properties** dialogue is displayed.

2. Select the **Run Data Colour** tab.

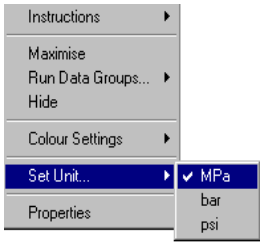
3. Click on the **Text** button or **Background** button and select a new colour. Click on **OK**. The result of the colour change is displayed in the tab.

4. Make further adjustments to the colours as appropriate.

5. Click on **OK** to close the dialogue and apply the changes.

Run data pressure units

If the Pressure data is displayed in the Run Data window you can set the displayed units.



1. Left click on the **Pressure** data to display the shortcut menu.

2. Select **Set Unit** and then the appropriate unit, either **MPa, bar** or **psi**, from the menu cascade.

   The selected unit is now displayed.

Run data manual instructions

Some strategies directly link specific manual instructions to the displays in the **Run Data** window. This is indicated by a double arrow in (**>>**) a display. A particular display can have one or more instructions attached to it. In cases where there is more than one instruction, one of the instructions is the main instruction.

- To view the instruction, or main instruction if there is more than one, double click on the display with the left mouse button. This opens the manual instructions dialogue for the instruction. Execute the appropriate manual commands.

  For more details on using manual instructions, please see “Manual instructions” on page 160.

- To select an instruction with the short-cut menu, click on the display with the shortcut menu and select **Instruction**. A cascade menu shows the specific manual instruction(s).

  Click on an instruction to select it. This opens the manual instructions dialogue in which you can execute the appropriate command.

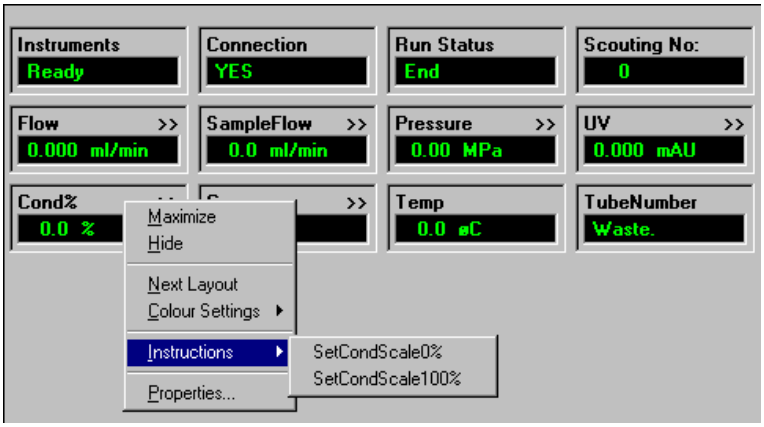


Fig 6-6. Selection of Run Data window showing individual displays with the double arrows (>>) indicating linked manual instruction(s). In this example the specific instruction(s) are shown in the short-cut menu for the Cond% display.

6.2.3 Curves

The curves window displays monitor signal values graphically.

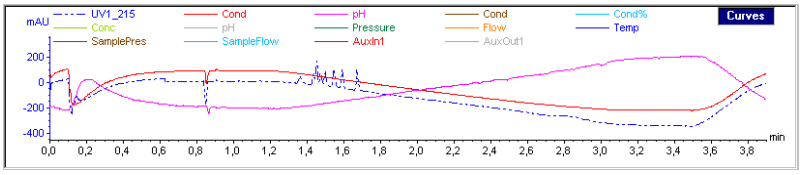


Fig 6-7. The Curves window.

To select the curves to be monitored on the screen:

1. Select **View:Properties** or select **Properties** from the shortcut menu. The **Properties** dialogue is displayed.

2. Select the **Curves** tab.

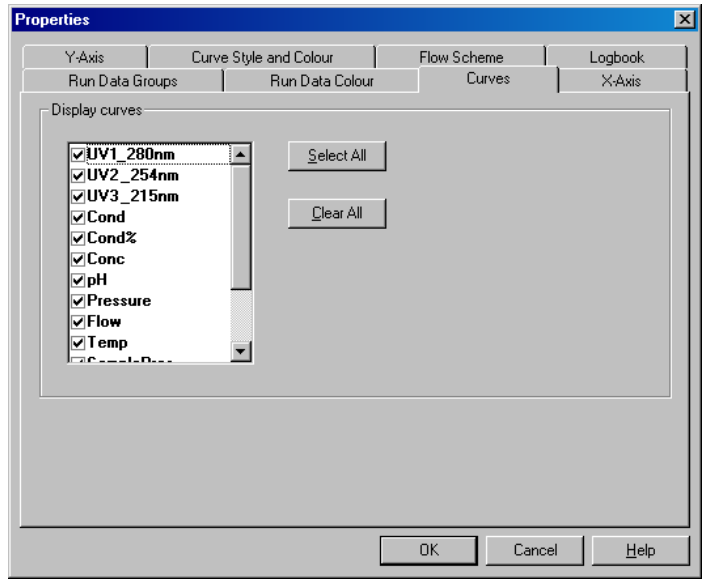


Fig 6-8. Properties dialogue, Curves tab.

3. Select the curves to be displayed from the list or click on **Select All** if you want to view all of the available curves. Note that curves will only be shown for components present in the chromatography system. To clear the selection, click on **Clear All**. The curves in this list are those for which **Store** is set to **On** in the system settings (see the UNICORN 4.0 Administration and Technical manual) together with any reference curves defined in the method.

Note: Fraction marks, injection marks and set marks will always be shown and are not curves in the list.

4. Click on **OK**.

Vertical cursor line

To display a vertical cursor line select **Marker** from the short-cut menu. Drag the cursor line with the mouse. Where the line bisects the curve, the X-axis and Y-axis values are displayed at the top right corner of the window.

Changing the curve colours and styles

The curves window displays graphs for the selected curves in different colours, with any reference curves included with the method as dashed lines. The curve colours and styles can be changed:

1. Select **View:Properties** or select **Properties** from the shortcut menu menu. The **Properties** dialogue is displayed.

2. Select the **Curve Style and Colour** tab.

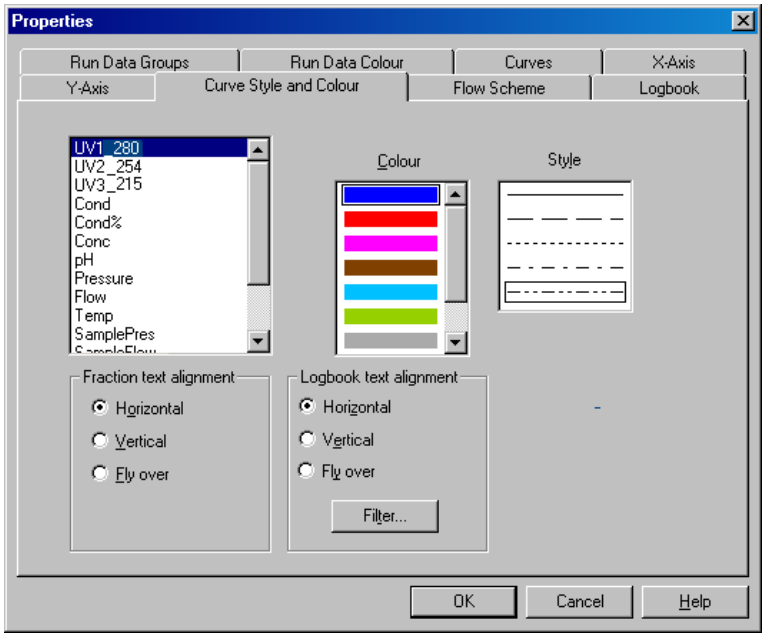


Fig 6-9. Properties dialogue, Curve Style and Colour tab.

3. Select a curve from the list and then select an appropriate colour and style.

4. Select the **Fraction text alignment** as desired.

5. Select the **Logbook text alignment.** It is possible to filter the type of information overlaid on the curve window by clicking on the **Filter** button. Select the information by pressing the radio buttons and set the maximum block depth. Click on **OK** to return to the **Curve style and Colour** tab.

Fig 6-10. The Filter logbook dialogue

5. Click on **OK** to save the Curve style and colour settings.

Changing the scale of Y-axis

The Y-axis is automatically scaled for each of the curves. To fix the scale of individual curves:

1. Select **View:Properties** or select **Properties** from the shortcut menu. The **Properties** dialogue is displayed.
2. Select the **Y-Axis** tab.

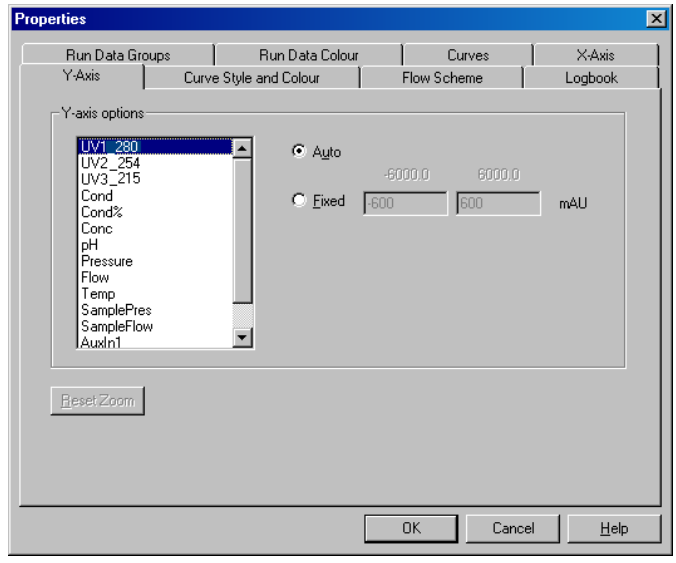


Fig 6-11. Properties dialogue, Y-Axis tab.

3. Select the appropriate curve and click on the **Fixed** button. Enter a minimum and maximum range in the fields within the specified limits.
4. Repeat steps 2 to 3 for other curves.
5. Click on **OK**.

Values on the Y-axis apply to the curve with the same colour as the axis markings. Click on the legend to get the correct Y-axis.

Changing the scale of X-axis

Click on the X-axis to switch the display between time and volume units. (The run is controlled according to the time/volume base defined in the current block, regardless of the base in the curves display).

Alternatively, select the X-units in the **X-Axis** tab of the **Curve Properties** dialogue.

You can also set the viewed portion of the total run.

1. Select **View:Curve Properties** or select **Properties** from the shortcut menu. The **Properties** dialogue is displayed.
2. Select the **X-Axis** tab.
3. Select the appropriate base, **Time** or **Volume**.

Note: Curves are collected in time and recalculated for display in volume. Thus, the resolution of the two bases may appear slightly different.

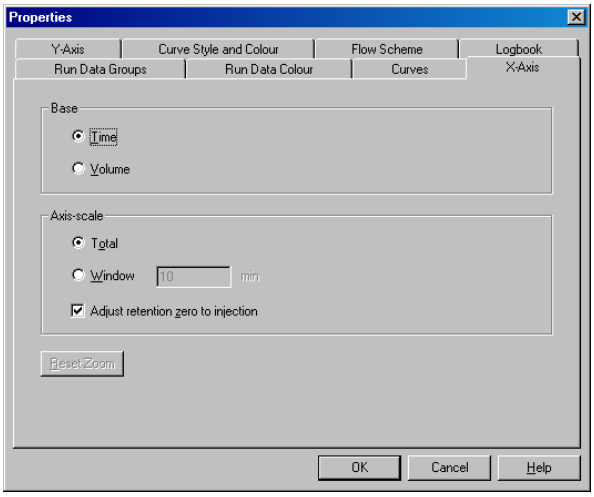


Fig 6-12. Properties dialogue, X-Axis tab.

4. Select the appropriate **Axis scale**, either **Total** or **Window**. The **Total** option will show the curves as far as they have come in the run. The **Window** option allows you to set the portion of the total window to be displayed, either in minutes or ml depending on the selected base. The **Adjust retention to zero** box, which influences the curve view and records in the logbook, is checked by default.

5. Click on **OK**.

The zoom function

To zoom in on a selected region of the curve window:

1. Press down and hold the left mouse button and drag a rectangle out on the screen to encompass the area to be viewed.

2. Release the mouse button. The display is now zoomed in on the selected area.

3. Repeat the process for further magnification of selected areas.

You can reduce the scale of the zoom in function in two ways, either:

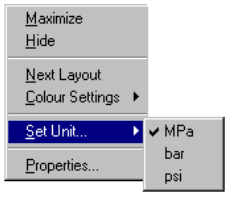
- reverse each zoom in action a step at a time by displaying the shortcut menu and selecting **Undo Zoom**, or,
- reverse all of the zoom in actions to the default scale setting by displaying the shortcut menu and selecting **Reset Zoom**

Viewing hatch marks

You can display hatching in the curve window by selecting **Hatch** from the shortcut menu.

Selecting curve pressure units

If the **Pressure** curve is displayed in the curves window you can set the displayed units.



1. Click on Pressure in Run Data to display the shortcut menu.

2. Select **Set Unit** and then the appropriate unit, either **MPa, bar** or **psi**, from the menu cascade.

   The selected unit is now displayed on the Y-axis.

Alternatively:

1. Select **Properties** from the shortcut menu.

2. In the displayed **Properties** dialogue select the **Y-Axis** tab.

3. Select the **Pressure** curve and then the appropriate pressure unit radio button. Click on **OK** to implement the change.

Selecting the text alignment

You can select the way that text is aligned for the logbook and fraction curve.

1. Select **Properties** from the shortcut menu.

2. In the displayed **Properties** dialogue select the **Curve Style and Colour** tab.

3. Select the **Logbook** or **Fraction** curve as appropriate.

4. Select the appropriate **Text alignment** option from **Horizontal**, **Vertical** or **Flyover**. Flyover displays the text if you place the mouse pointer over the generated mark. Click on **OK** to implement the change.

6.2.4 Flow scheme

The flow scheme is a graphical representation of the chromatography system. During a run, the flow scheme displays open flow path(s) in colour and monitor signals with numerical displays. The flow scheme thus shows the current status of the run at a glance.

Stretching a flow scheme

The flow scheme can be stretched to fit the screen by selecting **Stretch** from the shortcut menu. Alternatively, select the **Properties** option and the **Flow scheme** tab is shown in the **Properties** dialogue. Check the **Stretched** box to display a stretched view of the window.

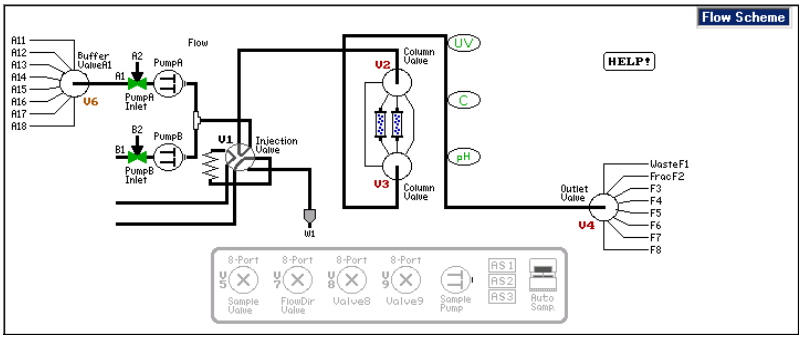


Fig 6-13. The flow scheme for a run.

Flow scheme manual instructions

Some strategies directly link specific manual instructions to the components in the flow scheme window. The components in the flow scheme that are associated with instructions are indicated with double arrow (**>>**). A particular component can have one or more instructions attached to it. In cases where there is more than one instruction, one of the instructions is the main instruction.

- To view the instruction, or main instruction if there is more than one, double click on the component with the left mouse button. This opens the manual instructions dialogue for the instruction. Execute the appropriate manual commands.
  For more details on using manual instructions, please see "Manual instructions" on page 160.
- To select an instruction using the shortcut menu, click on the component with the right mouse button and select **Instruction** from the shortcut menu. A cascade menu shows the specific manual instruction(s).

  Click on an instruction to select it. This opens the manual instructions dialogue in which you can execute the appropriate command.

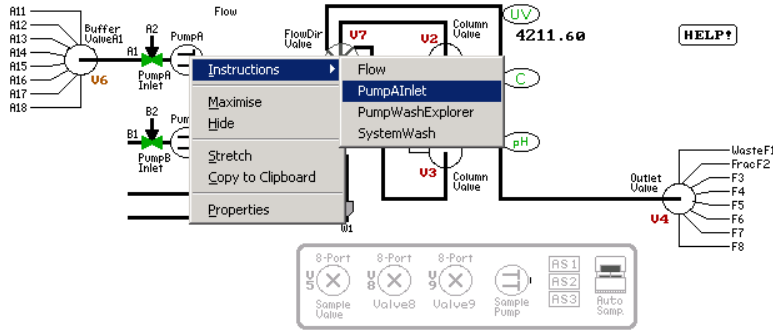


Fig 6-14. Selection of Flow scheme window showing components with manual instruction(s). In this example the specific instruction(s) are shown in the shortcut menu for the Injection Valve.

6.2.5 Logbook

All actions (including method start and end, base instruction, method instructions and manual interventions such as **Pause** or **Hold**) and unexpected conditions such as warnings and alarms are logged for every run, with date, time and current username where appropriate. (The date and time are taken from the system clock in the PC.) The logbook thus provides complete history of any given run. The log is saved in the result file.

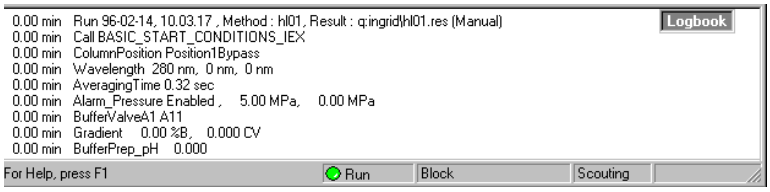

0.00 min Run 96-02-14, 10.03.17 , Method : hl01, Result : q:ingrid\hl01.res (Manual)
0.00 min Call BASIC_START_CONDITIONS_IEX
0.00 min ColumnPosition Position1Bypass
0.00 min Wavelength 280 nm, 0 nm, 0 nm
0.00 min AveragingTime 0.32 sec
0.00 min Alarm_Pressure Enabled , 5.00 MPa, 0.00 MPa
0.00 min BufferValveA1 A11
0.00 min Gradient 0.00 %B, 0.000 CV
0.00 min BufferPrep_pH 0.000
Logbook
For Help, press F1
Run
Block
Scouting

Fig 6-15. The logbook panel for a running method.

The logbook window can autoscroll to display the latest entries if you select the **Autoscroll** function from the shortcut menu or else check the **Autoscroll** option in the **Properties** dialogue.

6.3 Manual control

6.3.1 The toolbar

The toolbar at the top of the System Control workspace contains a set of buttons for starting and stopping the run (Manual Direct Commands Toolbar), accessing documentation (Windows toolbar) and locking the system (System toolbar). The toolbars can be shown and hidden by clicking on **View:Toolbars** and checking the relevant boxes.

Fig 6-16. The toolbar in the System Control workspace.

Manual direct commands toolbar

Run Starts a run when the system is in **End** state and a method is loaded.

Hold Suspends execution of a method, but continues to pump liquid at the current flow rate and eluent concentration settings. Accumulated time and volume continue to be incremented.
Any method instructions which are set to the time/volume when **Hold** is pressed are executed. Later method instructions are not executed until **Continue** is pressed.

Pause — Behaviour on **Pause** is strategy dependent. **Pause** suspends execution of a method and stops all pumps so that the system comes to a standstill. For ÄKTA design systems, valves remain in the position they were in before the pause. Accumulated time and volume is not incremented during **Pause**.

Any method instructions which are set to the time/volume when **Pause** is pressed are executed. Later method instructions are not executed until **Continue** is pressed.

Continue — Resumes execution of a paused or held method.

End — Terminates method execution and puts the system into **End** state.

These commands can also be located under the System Control **Manual** menu.

The available buttons in System Control are dependent on the control status of the connection:

Status	*Available buttons*
End	**Run**
Running	**Hold**, **Pause**, **End**
Manual	**Run**, **Pause**, **End**
Hold	**Pause**, **Continue**, **End**
Method pause	**Hold**, **Continue**, **End**
Manual pause	**Run**, **Continue**, **End**

Windows toolbar

The Windows toolbar has three buttons:

Opens a dialogue for choosing which window panels to display. Clicking on this button is equivalent to choosing the menu command **View:Window**.

Opens the documentation pages. Run notes may be entered in the **Notes** tab and settings may be changed. Other tabs are displayed for information only. Clicking on this button is equivalent to choosing the menu command **View:Documentation**.

Opens the properties pages. Clicking on this button is equivalent to choosing the menu command **View:Properties**.

System toolbar

The System toolbar has two functions:

Disconnect/Connect System. It is possible to disconnect (left icon) the system and leave it in locked or unlocked state. Use the connect icon (right) to connect the system.

Leave/Take Control of the System. It is possible to leave (left icon) the system in locked or unlocked state. Use the Take Control icon (right) to take control of the system.

Status bar

The status bar also displays a text message indicating the connection status of the window:

Controlled by: <user> The indicated user has a control mode connection to the system. Other users may establish a view mode connection.

Locked by: <user> The indicated user has left the system in a locked state. Users who can supply the required password may unlock the system and establish a connection. Note that the password is case sensitive.

Note: It is possible to unlock with the "lock" password or with the UNICORN logon password. If using the UNICORN logon password, the user must have the **Unlock systems** access rights. The "lock" password is the password entered by the user who locked the system and is case sensitive.

System is available Any user may establish a connection.

6.3.2 Manual instructions

The chromatography system can be controlled with manual instructions issued from the **Manual** menu. The available instruction options are dependent on the strategy, and instructions for the components defined for the system are displayed. To save the results of a manual run, issue the instruction **Record_on** (in the **Other** instruction group) at the beginning of the run. UNICORN will prompt for a result file name at the beginning of the run. When you choose to run the system manually - as opposed to a **Method run** - the results are stored in a folder called **Manual Runs**.

The **Manual Runs** folder stores the ten most recent results from your manual runs. To more permanently save a result from a manual run, you need to move or copy it to another location.

The **Manual** menu opens a dialogue similar to the text instruction box in the **Method Editor**.

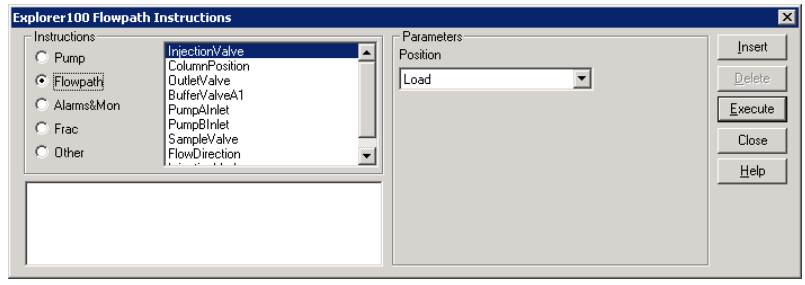


Fig 6-17. The Manual instruction dialogue.

The name of the connected system is displayed in the title bar of the dialogue. Available instructions are determined by the strategy and selected optional components for the connected system.

Manual instructions are entered in the same way as method instructions from the dialogue in the Method Editor. The **Insert** button places the current instruction in the list at the bottom left of the dialogue. Clicking on **Execute** executes all instructions in the list at the same time, or executes the currently marked instruction if the list is empty. Note that although all instructions are executed simultaneously, some (for example gradient and fraction instructions) may take some time to complete in the liquid handling module.

The **Delete** button deletes the selected instruction from the current list. One instruction can be deleted at a time.

If you close the dialogue by clicking on the **Close** button without choosing **Execute**, commands in the list will not be executed and will be deleted from the command list. Manual instructions can also be issued while a method is running. A manual setting applies until the next method instruction of the same type is executed (e.g. a manual **Flow** instruction will set the flow rate until the next **Flow** instruction in the method is executed). Manual instructions that you issue during a method are recorded in the logbook for the method run.

6.3.3 Alarms and warnings

The system settings determine the acceptable limits of monitor signals during a run. The limits can also be set for the current run using an instruction in the method. Limits set with a method instruction override the limits set in system settings. If these limits are exceeded in a run, a warning (W) or alarm (A) dialogue with a message is displayed on the screen.

- The run continues if a warning is issued.
- An alarm pauses the system.

Warnings and alarms are displayed regardless of the activity currently in progress in UNICORN: you will be notified of an exceeded limit in a running system even if you are developing a method, evaluating data or monitoring a run on a different system. Warnings and alarms are also recorded in the logbook for the run.

In a network installation alarms and warnings are displayed on the controlling station and all stations viewing the system. An alarm can be acknowledged only from the computer connected in control mode: alarms are displayed but cannot be acknowledged on computers connected in view mode.

6.4 If communication fails

If the results of a run were to be stored on a server or another location and there is network communication failure during a run that has been started from a remote station, the run will continue and the results will be saved in the **Failed** folder on the local station. A control mode connection can be established on the local station to control the running system. See the Security section of the UNICORN Administration and Technical manual for more details.

6.5 Maintenance

Some strategies, such as for the ÄKTA family, support the possibility to view system information for the components in a chromatography unit and to define warnings on the components for maintenance purposes.

6.5.1 Viewing system component information

To view the maintenance functions for a maintenance-supported chromatography system:

1. Select **System:Maintenance.** The **Maintenance manager** dialogue is displayed. The **Info** tab is shown by default and UNICORN takes a few moments to scan the connected chromatography system for the components present.

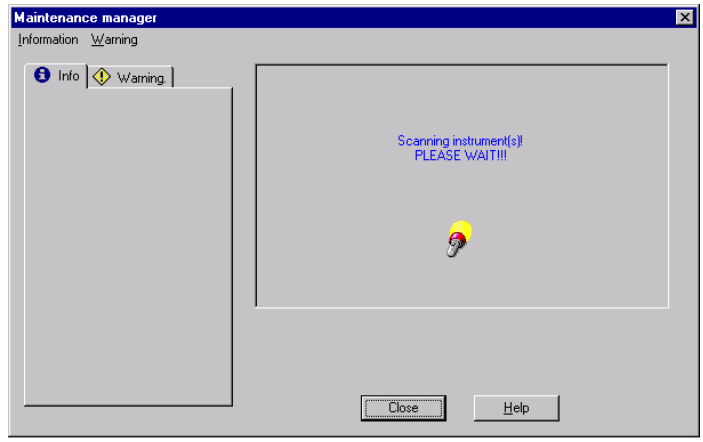


Fig 6-18. Maintenance manager dialogue, Info tab.

When UNICORN has completed its scan all system components are displayed.

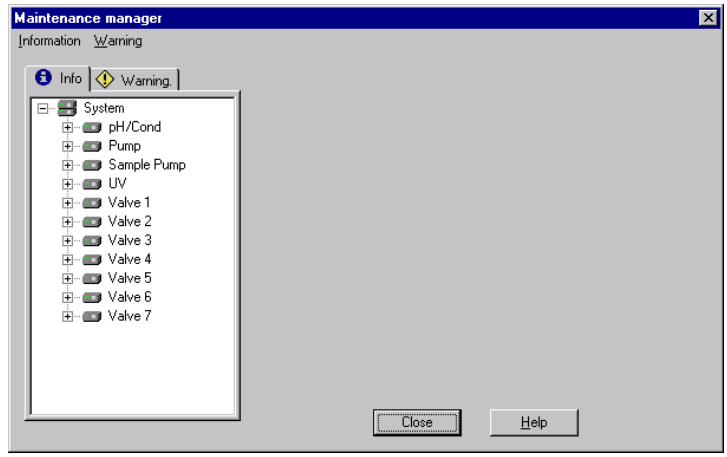


Fig 6-19. Maintenance manager dialogue, Info tab with system components displayed.

2. To view the information about a specific component, click on it. The information can be viewed on the right-hand side of the tab.

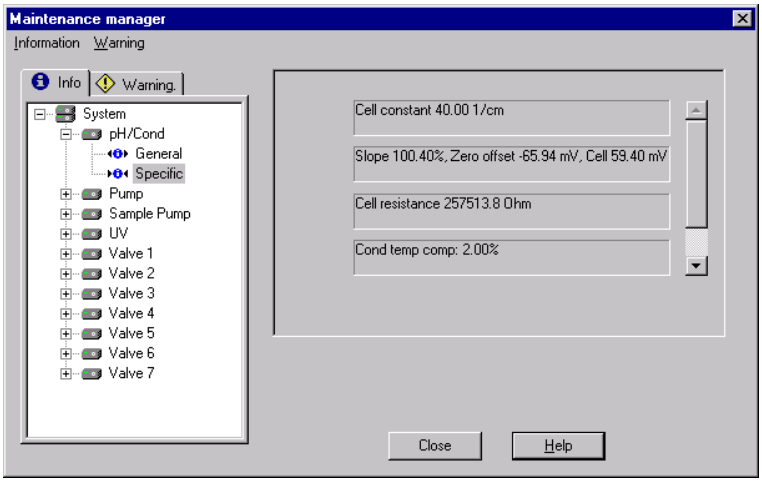


Fig 6-20. Maintenance manager dialogue, Info tab with information displayed for pH/Cond cells.

You can select to view **General** information, which contains information such as serial number, version number etc., or **Specific** information, which contains information such as how long the component has been used, how many hours for example a pump has run etc.

6.5.2 Setting up maintenance warnings

To set up a maintenance warning:

1. Click on the **Warning** tab. All of the system components are displayed.

2. Click on a specific component for which you want to set up a warning and select **Warning:New** from the menu. Alternatively, click with the shortcut menu on the specific component and select **New** from the displayed menu.

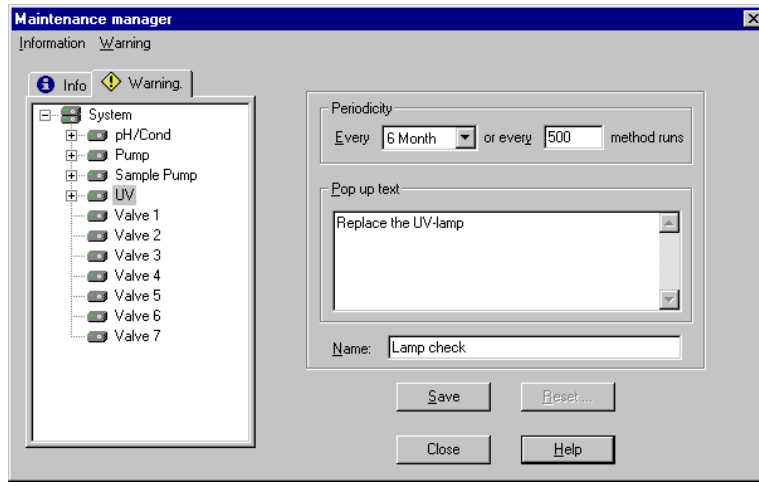


Fig 6-21. Maintenance manager dialogue, Warning tab.

3. Enter the appropriate values for **Periodicity,** enter the desired **Pop up text** and enter a **Name** for the warning type.

4. Click on **Save** to save the warning. The new warning is added to the specific component.

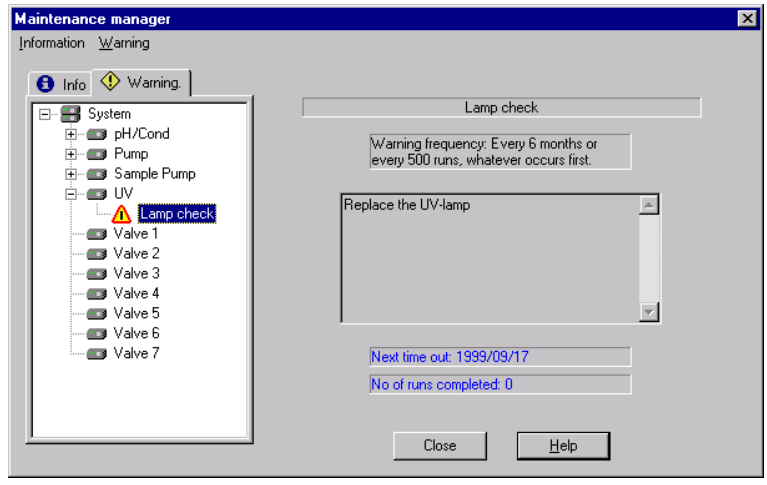


Fig 6-22. Maintenance manager dialogue, Warning tab with a defined warning set up for the UV lamp.

5. Repeat the process for setting up warnings for the same component or other components in the list.

6. Click on **Close** when you have made the appropriate selections.

6.5.3 Viewing and resetting the warning parameters

A counter is set up linked to the **Periodicity** that you defined for a new warning message (see above). By entering the edit warning mode you are able to zero the warning parameters. You can do this by selecting the warning in the **Warning** tab followed by selecting **Warning:Edit** from the menu. Alternatively, click with the shortcut menu on the warning and select **Edit** from the displayed menu. In the edit mode click on the **Reset** button, which displays the **Reset parameters** dialogue.

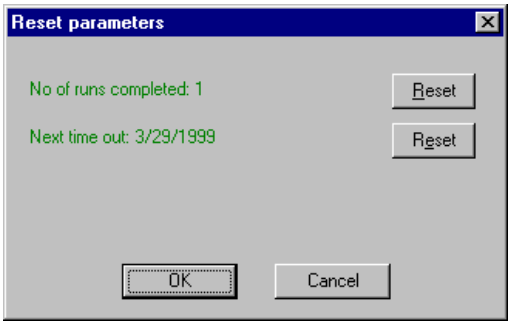


Fig 6-23. Reset parameters dialogue.

You can zero specific counters by clicking on the associated **Reset** button in the dialogue.

6.5.4 Getting a warning

When you end a method using a chromatography system for which maintenance warnings have been set up, a warning message will be displayed once the specific **Periodicity** parameter has been reached.

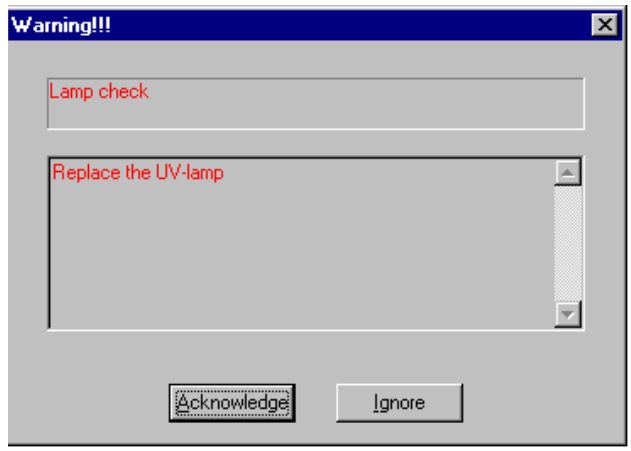


Fig 6-24. Warning dialogue for a lamp check.

Select **Acknowledge** if you have corrected the problem in the **Warning** dialogue. Select **Ignore** if no correction has been made. If **Ignore** is selected, you will be reminded about the unresolved problem(s) at a later stage.

7 Scouting

Scouting is a facility for automatically repeating a run with systematic variation of one or more parameters. Some typical situations where scouting is useful are:

- screening for the best column
- finding the optimal pH
- testing column capacity (sample volume)
- finding the optimal flow rate for binding and elution
- optimising gradient length and slope
- optimising step gradients

This chapter describes how to set up and use scouting schemes in UNICORN.

7.1 Setting up scouting

Scouting schemes are defined as part of the Run Set-up in the Method Editor (see "Scouting" on page 71). Any parameters (including breakpoints) can be scouted provided that they can be defined as variables in the method. To set up a scouting scheme:

1. Create a method by selecting a method template or Wizard. If a method is created without using a method template or Wizard, define appropriate variables in the method.

2. Open the **Scouting** tab in Run Set-up.

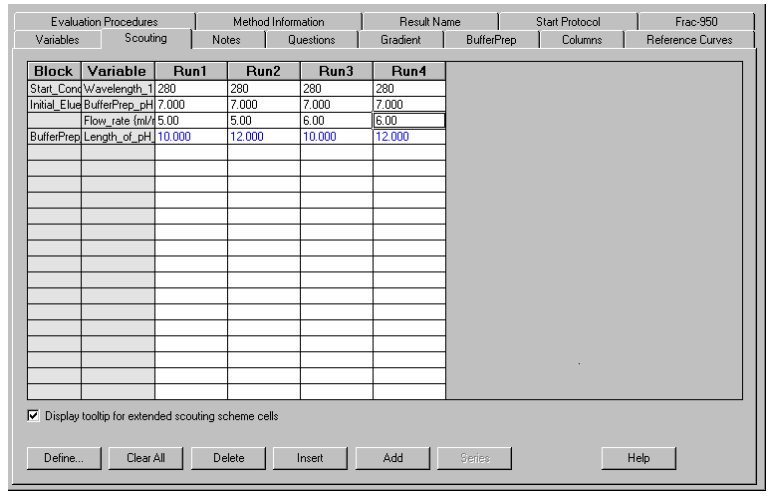


Fig 7-1. The Scouting tab in Run Set-up showing a scheme for testing flow rate.

3. The first time the **Scouting** tab is opened no variables are selected and the **Define Variables** dialogue is automatically displayed. Otherwise, click on **Define** and choose the variables you wish to scout from the list.

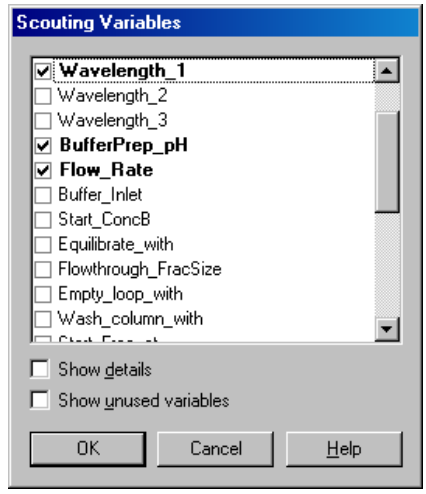


Fig 7-2. The dialogue for selecting scouting variables.

By default, no variables in the method are selected. Select only the variables you wish to scout.
Checking the **Show details** checkbox will show variable details on the **Variables** tab in Run Set-up. Similarly, the **Show unused variables** checkbox will display variables that are present in blocks but that are not used in the method.

5. Make any changes you require in the variable values. Use the scroll bar at the right-hand side of the tab if the variables do not fit onto one screen. Certain variables use the drop-down menus in the **Variable** column for selecting options. Variable values in blue can be switched from single position name (e.g., ON, OFF) to variable range by clicking on the value with the right mouse button.

6. Click on the **Add** button to copy the values from the previous run, and change variable values as required.

7. Repeat this procedure until you have defined all the runs you require. Up to 99 runs can be defined for scouting. You can delete and insert run columns using the **Delete** and **Insert** buttons respectively.

   Series of runs for vial number are created by clicking on the variable cell in the run you wish to start the series and then by clicking on the **Series** button. Enter ranges vial number in the **Insert series** dialogue and press **OK**.

8. If you want to exclude runs from the default scouting scheme, right-click on the **Run** button at the top of the **Variable** column to toggle between **Run** and **Excluded**.

9. Open the **Start Protocol** tab in Run Set-up. Check the **Scouting** box if you want the **Scouting** page to be displayed at the start of the run (recommended). This allows the operator to adjust the **Run/ Excluded** status for individual runs and the values for scouting variables before the method starts. If this box is not checked, running the method will perform scouting as defined in the method.

   If the **Variables** box is also checked, the start protocol will display the **Variables** page as well as the **Scouting** page. Values for variables defined for scouting are, however, grey in the Variables tab and cannot be changed there.

   On the **Start Protocol** tab, in the **Scouting start protocol** field, check the **First run only** radio button if you want the start protocol to be displayed only before the first run. If this button is selected, the settings entered in the start protocol for the first run will apply

throughout the run, and the scouting series will be performed automatically without user intervention. Select **All runs** if you want the start protocol to be displayed before each run in the scheme, giving the operator an opportunity to change variable values or e.g. fill the sample loop before each run. The operator must then press **Start** before each run in the scouting scheme.

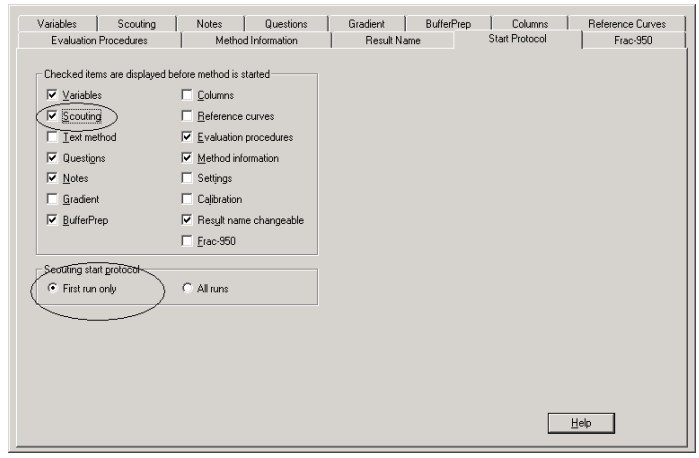


Fig 7-3. Settings on the Start Protocol tab in Run Set-up determine whether the Scouting page will be displayed at the beginning of scouting runs.

10. Open the **Result Name** tab in Run Set-up and enter a name for the scouting folder. Change the path for the result file if required. The scouting results will be saved in a folder on the result file path, named according to the specified scouting folder name with a three-digit serial number.
11. Save the method.

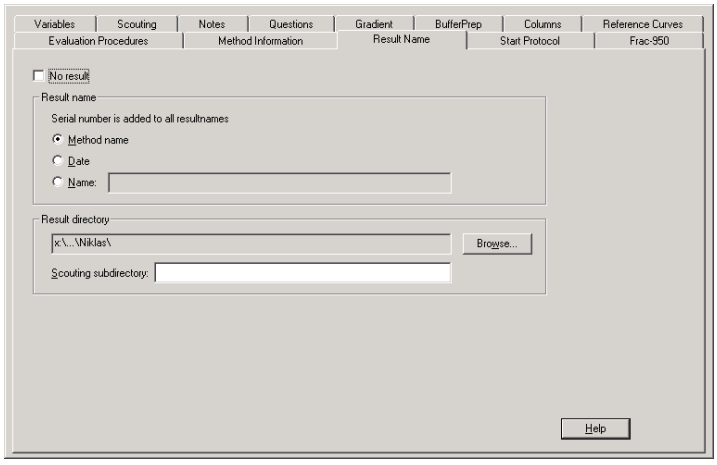


Fig 7-4. Settings under Result Name on the Result tab in Run Set-up determine the name of the folder where scouting results will be stored.

7.1.1 Scouting variables

The variables that appear in the scouting scheme are usually a subset of those on the **Variables** tab of Run Set-up. However, while the default values in the method can be set either on the **Variables** tab or in the text instructions (see "Method variables" on page 66), values in the scouting scheme can only be set on the **Scouting** tab. Changing variable values in the scouting scheme does not change the values on the **Variables** tab or in the text instructions. Values for variables selected for scouting are grey on the **Variables** tab and cannot be changed there.

7.1.2 Scheme set-up

Each run column in the scouting scheme represents one potential run through the method. The runs which will actually be performed are defined by the status of the **Run/Excluded** button at the top of each column. Runs can be included or excluded by clicking with the right-mouse button on the top of the **Run** columns.

7.1.3 Start protocol settings

If the **Scouting** box in the **Start Protocol** tab is not checked, the scouting scheme will run automatically as defined in the method. (We recommend that the box is always checked for a scouting method. There is otherwise no indication for the operator at the start of the run that the method actually performs several runs).

7.2 Running scouting

To perform a scouting run:

1. Start the method (see "Starting a method" on page 141). The start protocol will display the scouting scheme as defined in the method (assuming that the **Scouting** box is checked in the start protocol definition, see above).

2. Check through the settings for the scouting scheme, and if required:

   - change variable values for the runs
   - using the right mouse button, click on the **Run** column to toggle the run status between **Run** and **Excluded**.

   If the start protocol is displayed at the beginning of each run, you can adjust the settings for the current and subsequent runs at the beginning of every run. If the start protocol is not displayed at the beginning of each run, the sequence of runs will be performed automatically according to the settings entered in the start protocol for the first run.

3. Work through the rest of the start protocol, then press **Start**.

The results of a scouting run are saved in a special scouting folder as defined in the **Results** tab of the start protocol. Within the folder, each run is saved in a separate result file named according to the usual naming rules (see "Result Name" on page 91).

Note: If the start protocol is displayed for each run in a scouting scheme, you may be able to change the result file name during scheme execution.

7.2.1 Changing scouting settings during a run

At any time during a run, you can click on the **Documentation** icon in System Control and change the scouting settings in the **Scouting** tab for runs which have not yet been started. Settings for the run which is currently in progress cannot be changed.

You can use this feature to adjust settings for scouting even if the start protocol is not displayed at the beginning of each run.

7.2.2 Adding scouting runs during a run

As long as the last run has not yet started it is possible to add new scouting runs. To do this click in one of the variable fields in the first empty column. The values from the previous run will be copied into the new column and you can change the variable values as required.

Repeat this process until you have defined all the runs that you wish to add.

7.3 Scouting results

7.3.1 Viewing results

Each result file in the scouting result folder represents the results of one scouting run, and can be viewed in the same way as result files from ordinary runs (see Chapter 9). The **Scouting** tab in the documentation saved in each scouting result file shows the whole scouting scheme, with the values for the current run displayed in a cell with a yellow background.

Note: Any changes made to the variable values during a run will only be recorded in the result files for runs after the changes were made.

7.3.2 Comparing runs

To compare chromatograms in results from different runs in a scouting series open a result file and choose **File:Multifile Peak Compare** or click on the Multifile Peak Compare Wizard icon (see "Multifile Peak Compare" on page 207).

7.3.3 Printing results

Scouting results can be evaluated and reports can be generated in the same way as for other result files (see Chapters 9 and 10). The following settings in **File:Report** in the Evaluation module are important for scouting:

Documentation:Scouting includes the complete scouting scheme, but does not indicate which run generated the particular result file.

Documentation:Variables prints the variable values used during the particular run.

Edit:Chromatogram Layout Box, **Header** tab **Scouting Variables** option prints the scouting variable values for the particular run as part of the diagram header.

For reports generated from scouting runs, always make sure that **Scouting Variables** is selected in **Edit:Chromatogram Layout** box, **Header** tab so that the scouting variable values for the particular run are included.

7.4 Scouting examples

Below are some typical examples of scouting, illustrating the way scouting schemes can be set up for different purposes.

7.4.1 Scouting sample volume

Sample volume scouting is applicable to both adsorption and gel filtration techniques. Superloop can be filled with sample and the sample volume varied with the **Empty_loop_with** variable.

Block	Variable	Run1	Run2	Run3	Run4
Sample_Injection	Empty_loop_with {ml}	2.500	2.750	3.000	3.250

Fig 7-5. Scouting settings for Sample_Injection.

7.4.2 Screening different columns

Scouting can be used to screen different column types for an application.

For screening different columns, a suitable scheme may include the variables:

- **Column**
- **Column_position** (switches the ColumnValves to the chosen column)
- **Flow_rate**

Block	Variable	Run1	Run2	Run3	Run4
Main	Column {ml}	10.000	0.100	0.100	0.100
Start_Instructions	Column_Position	Position1By	Position2	Position3	Position4
	Column_PressureLimit {	4.00	4.00	4.00	4.00
	Wavelength_1 {nm}	280	280	280	280
Flow_Rate	Flow_Rate {ml/min}	2.00	4.00	6.00	8.00

Fig 7-6. Screening different columns, with facilities for changing the Column, Column_position and Flow_rate for each column.

7.4.3 Scouting continuous gradients

Scouting continuous gradients involves finding the best final concentration and gradient length for elution of the product from a given column. These two parameters are set as variables.

Block	Variable	Run1	Run2	Run3
Gradient_Segment_1	Target_ConcB_1 {%B}	100.00	100.00	100.00
	Length_of_gradient_1 {CV}	5.000	10.000	20.000

Fig 7-7. Scouting for optimal gradient slope and length.

7.4.4 pH scouting

With **BufferPrep** it is possible to vary the pH automatically in the different runs.

Block	Variable	Run1	Run2	Run3
Initial_Eluent_Conditions_BP	BufferPrep_pH	3.000	4.000	5.000

Fig 7-8. Scouting for optimal pH.

8 MethodQueues

MethodQueues provide a means for linking several methods together, on the same or different systems. For example, if a system wash procedure is programmed in a separate method, it can be linked in a MethodQueue to a series of different process methods, ensuring that the same wash procedure is used before every process. Alternatively, the product of a separation on one system might form the starting material for a separation on the next, allowing fully automated multi-step processing.

Specific user authorisation is required to edit and run MethodQueues, separate from that required for editing and running methods.

8.1 Setting up a MethodQueue

8.1.1 Defining a MethodQueue

To create a new MethodQueue:

1. In Main Menu, click on the **MethodQueue** icon, or select either **File:New:MethodQueue** or **Tools:MethodQueue:New**. The **MethodQueue Editor** dialogue is displayed.

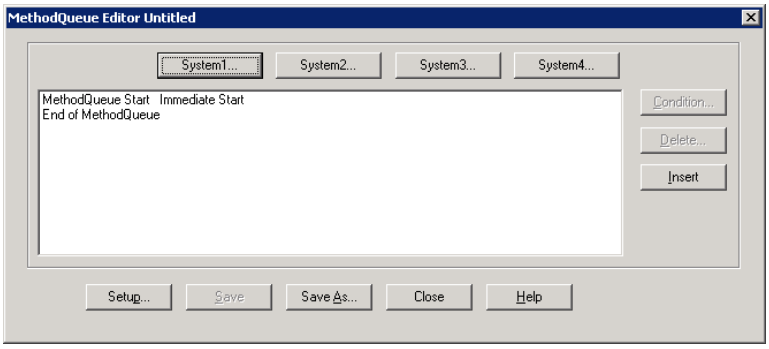


Fig 8-1. MethodQueue Editor dialogue.

2. To add a method to the MethodQueue list, select the **End of MethodQueue** instruction in the list.

3. Click on the appropriate **System #** button. The **Load MethodQueue** dialogue is displayed.

Note: The number of available **System #** buttons is dependent on the number of systems that were selected during installation of UNICORN.

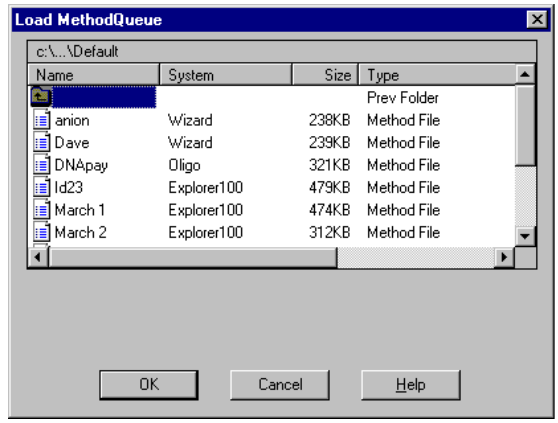


Fig 8-2. Load MethodQueue dialogue.

4. Use the dialogue to locate and select the required method. Click on **OK** or double click on the method file.

   The system type for the selected method, e.g. **Explorer 100**, is assigned to the selected **System #** button and the **System #** name on the button is replaced with the name of the system.

   A line is also inserted into the MethodQueue list before the previously selected **End of MethodQueue** instruction. The new line contains the name of the selected method.

5. To add more method steps click on the **Insert** button and repeat steps 3-4. Note that you must use a new **System #** button to add methods written for a different system type.

   For example, if you have designated **System 1** to methods for the **Explorer 100** system, then only methods written for Explorer 100 can be added with this button. If you want to add to the list methods written for, for example, Basic 10 then you must use another free **System #** button such as **System 2**. In this case, **System 2** will be assigned and renamed **Basic 10** and only methods written for this system can be added using this button.

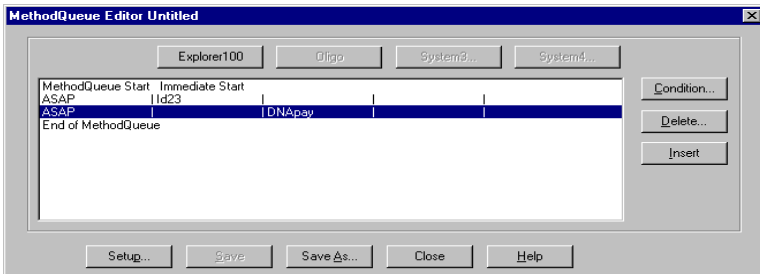


Fig 8-3. MethodQueue Editing dialogue with a method selected for an Explorer 100 system.

6. By default each method step will start as soon as possible (**ASAP**) after the completion of the previous method step. To set the time interval for starting a selected step click on the **Condition** button. In the **Condition** dialogue set the time when the step is to start. Click on **OK**.

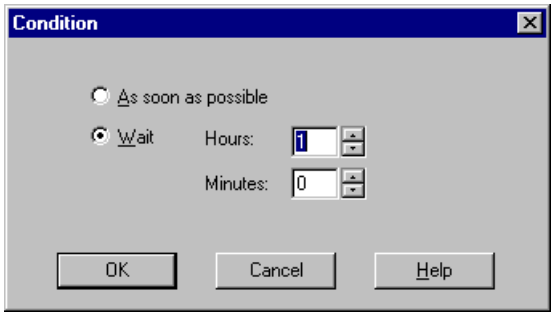


Fig 8-4. Condition dialogue.

The timing of MethodQueue steps performed on different systems can also be controlled by the **Ready** instruction (see "Method execution in MethodQueues" on page 181) in the method.

7. Click on **Setup** to define the starting time for the MethodQueue. **Immediate Start** sets the MethodQueue to start as soon as you request **Run** from the Main Menu. **Start time** sets the MethodQueue to start at a pre-set time of day up to one week after **Run** is requested. If no day is specified, the MethodQueue will start as soon as the pre-set time is reached (i.e. within the next 24 hours). Click on **OK** to complete the dialogue and return to the **MethodQueue Editor** dialogue.

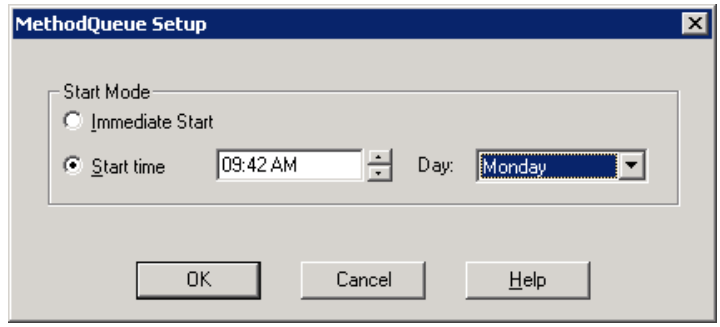


Fig 8-5. MethodQueue Setup dialogue.

Note: The MethodQueue setup time defines the starting time for the MethodQueue as a whole. Do not confuse this with **Condition**, which defines the relative starting time for a step within a MethodQueue.

8. Click on **Save** to save the MethodQueue. Enter a MethodQueue name in the dialogue.

Note: **Edit MethodQueue** authorisation is required to define a new MethodQueue.

8.1.2 MethodQueue folders and icons

MethodQueues are saved in a separate MethodQueue folder within the folder that you specified during the save. The MethodQueue folder is represented by a special icon in the **Methods** window of the main UNICORN menu.

MethodQueue folder.

Double-click on the MethodQueue folder icon to open it. A MethodQueue folder contains the MethodQueue definition and copies of all methods included in the MethodQueue.

MethodQueue definition (in MethodQueue folder).

It is important to realise that the MethodQueue works with copies of the original method files. If changes are made in the original method, these will *not* affect the method in the MethodQueue. To implement changes in a MethodQueue method, edit the method in the MethodQueue folder. Alternatively, edit the original method, then use

the MethodQueue editor to update the MethodQueue, replacing the old method with the changed version. It is a good idea to make sure that MethodQueue definitions always contain updated methods, to avoid confusion between different versions of method files.

8.2 Editing MethodQueues

To edit an existing MethodQueue, open the MethodQueue folder icon with the shortcut menu command, **Edit**. The **MethodQueue Editor** dialogue is displayed for the selected MethodQueue. **Edit MethodQueue** authorisation is required to edit a MethodQueue.

- To change the start condition or method name, select the line to be edited and click on the **Condition** or assigned system button respectively.
- To insert a new MethodQueue line after the currently selected line, click on **Insert**.
- To erase the currently selected line from the MethodQueue, click on **Delete**. In the dialogue box, check the system(s) for which the method is to be deleted.

8.3 Running a MethodQueue

Before starting a MethodQueue run, make sure that all systems used in the MethodQueue are connected with control mode connections and are in **End** status. The system associated with the first **System** button in the MethodQueue definition must be connected to System Control window 1, the second system to System Control window 2 and so on. The MethodQueue will not start unless all required systems are connected in control mode.

To start a MethodQueue from the Main Menu, select the MethodQueue icon and select **File:Run** or select **Run** from the shortcut menu. The MethodQueue will start in accordance with the conditions defined in the MethodQueue setup. You cannot start or end a MethodQueue from the System Control window.

Run MethodQueue authorisation is required to start a MethodQueue.

8.3.1 Method execution in MethodQueues

The start protocol for the first and each subsequent method step in the MethodQueue is displayed when the corresponding method is run. If you require unattended MethodQueue operation after the start of the first method step, make sure that subsequent method steps do not include a start protocol.

The **Condition** setting for each step in a MethodQueue determines the relative timing of the steps. If successive methods are run on the same system, the timing set in **Condition** applies from the completion of one method to the start of the next.

If successive methods are run on different systems, the **Ready** instruction in one method can be used to trigger the start of the next method, i.e with the **Ready** instruction you will be able to start the next method already before the current method has ended. The Condition setting then applies from the **Ready** instruction to the start of the triggered method. This is useful for example in situations where a method on one system prepares the starting material for the next, and then continues to wash the system:

System 1	System 2
Apply sample	
Elute	
READY----------	Apply sample
Wash	Elute
	.....

8.4 Displaying MethodQueues

Click on the Display running MethodQueue icon or choose **MethodQueue:Display running** to display pending and running MethodQueues. A pending MethodQueue is one for which **Run** has been requested, but which has not yet started either because the system is not available or because the Setup time has not been reached.

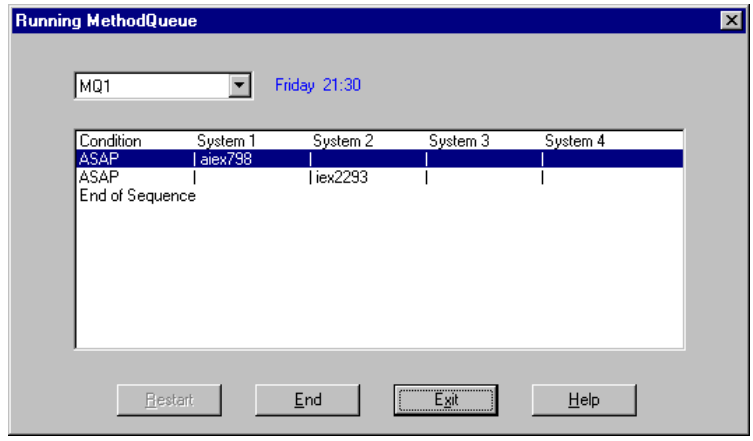


Fig 8-6. Displaying running and pending MethodQueues.

Use the list box at the top left corner of the dialogue to select a MethodQueue. The list shows pending and running MethodQueues.

For the selected MethodQueue, **Start at** shows the time when the MethodQueue is programmed to start. The actual time of start is shown for a currently running MethodQueue. The buttons in the MethodQueue display have the following functions:

Restart	Restarts the currently running MethodQueue if a start protocol has been terminated by Cancel.
End	Terminates a running MethodQueue after the current step. Any methods currently in operation will continue to run, and must be terminated with End in the System Control window if they are not to run to completion. Clicking on **End** for a pending MethodQueue deletes the MethodQueue from the pending list.
Exit	Closes the MethodQueue monitor panel.

9 Presenting results

A result file is automatically generated at the end of a run and contains a complete record of the run, including method, system settings, curve data and run log. The Evaluation Module offers extensive facilities for presenting and evaluating curve data.

This chapter describes how to:

- view the documentation from a run
- present the chromatograms and curves of your result file
- compare chromatograms and curves
- print reports

9.1 Opening a result file

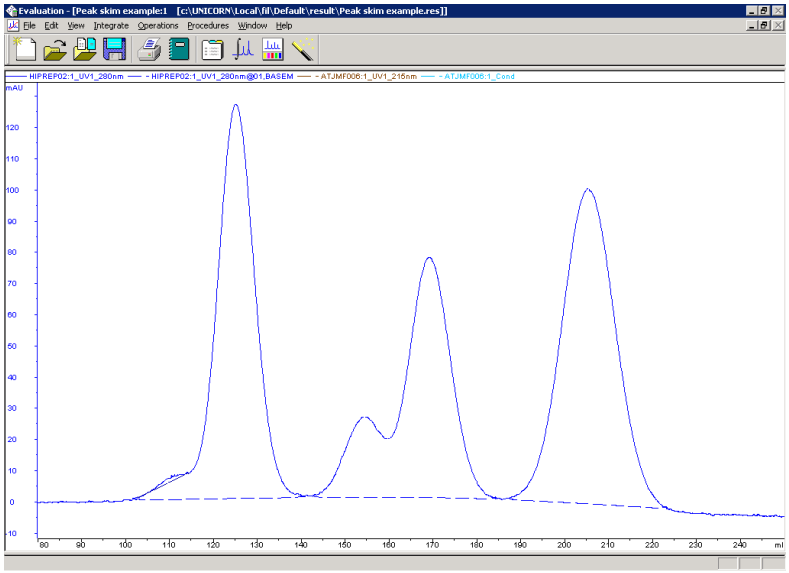


Fig 9-1. Evaluation module view.

To open a result file, either:

- double click on a result file icon in the **Results** window of the Main Menu,

- select a result file icon in the **Results** window of the Main Menu and select **File:Open**,
- click on the **Evaluation** icon in the Windows taskbar, select **File:Open** or click on the **Open** toolbar button, and select a result file from the **Open Result** dialogue,
- click on the **Evaluation** toolbar button in the Main Menu and select a result file from the **Open Result** dialogue.

All contents of the opened result file are transferred to the Evaluation workspace. By default, the chromatograms in a run are shown as opened windows. The chromatogram window on top is the active window. There is also a minimised **Temporary** chromatogram window.

9.1.1 Chromatogram

A chromatogram includes a number of curves that have been created during a run, such as UV, conductivity, pH, fraction marks, etc. The original raw data curves cannot be deleted or modified, although they can be used as the basis for evaluation procedures and subsequent creation of new curves. A chromatogram also contains the curves created and saved during an evaluation session. The default name for the first chromatogram in a result file is 1.

9.1.2 Temporary chromatogram

The **Temporary** chromatogram is essentially an empty chromatogram and is specific to the Evaluation module. Thus, curves can be copied into **Temporary** using **Edit:Copy:Curve** and comparisons and/or evaluations can be performed. This is particularly useful if you do not want to clutter up your original chromatograms with a large number of curves. It can also be used to keep blank run curves or curves to compare when opening different result files. Information contained within the **Temporary** chromatogram is automatically saved from one evaluation session to the next, but is not saved within the result files. Click on the window restore button or select **Windows:Temporary**. The contents of the temporary chromatogram can be removed by selecting **Edit:Clear Temporary Chromatogram**.

9.2 Basic presentation of chromatograms

This section gives directions on how to access result files and optimise the presentation of a chromatogram and its curves via the **Chromatogram Layout** dialogue. The last evaluation operation that is performed can be undone using **Edit:Undo**.

9.2.1 The chromatogram window

The chromatogram window is divided into three main views for header information, run curves and peak tables. The displayed areas for the views can be adjusted by dragging the borders with the mouse cursor between the views.

Viewing the curves

The first time a chromatogram window is opened and viewed, a default layout is applied to display all of the original curves. The default layout can be changed by the user (see "Saving and applying a layout" on page 195).

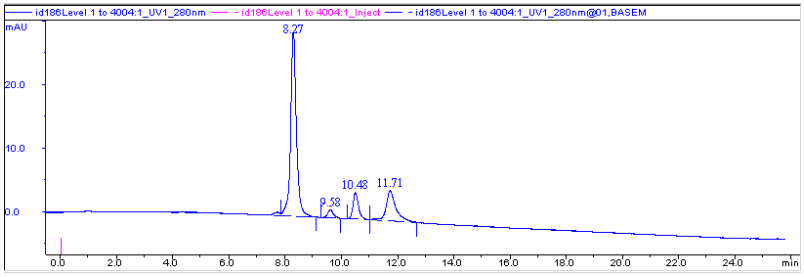


Fig 9-2. Displayed chromatogram in a newly opened result file.

Each curve is automatically assigned a default colour and style, with default information about each curve displayed in the key above the curves. This information includes the result file name, chromatogram name and curve name.

Each curve has a correspondingly coloured Y-axis. To choose the appropriate Y-axis scale, click on the Y-axis until the desired scale is displayed, or simply click on the name of the curve of interest.

Optimising the workspace

Chromatograms can be minimised in the desktop by clicking on the minimize button in a chromatogram window. Icons can be neatly arranged in the workspace by selecting **Window:Arrange icons**.

To restore a window, click on the restore button for the iconised window or select the chromatogram name from the **Window** menu. You can also maximise a chromatogram window to fit in the whole Evaluation desktop by clicking on the maximize button.

To view several chromatogram windows side by side select **Window:Tile**. Alternatively, **Window:Cascade** will stack all of the open windows like a deck of cards.

9.2.2 Shortcut menu

When viewing curves in the Evaluation module, the shortcut menu provides a quick alternative to menu commands. The following functions are available from the shortcut menu:

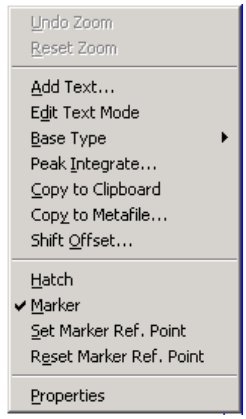


Fig 9-3. An example of a shortcut menu within the Evaluation module.

9.2.3 Opening the Chromatogram Layout dialogue

Most of the changes that you are likely to make regarding chromatogram presentation, are made in the **Chromatogram Layout** dialogue. This is opened in one of two ways:

- Place the mouse cursor in the chromatogram window and select **Properties** from the shortcut menu options. Note that the view from which you activate the **Properties** command determines the tab that is displayed in the **Chromatogram Layout** dialogue.
- Select **Edit:Chromatogram Layout**.

Note: You can apply any changes made in the **Chromatogram Layout** dialogue to all open chromatograms by checking the **Apply to all chromatograms** option.

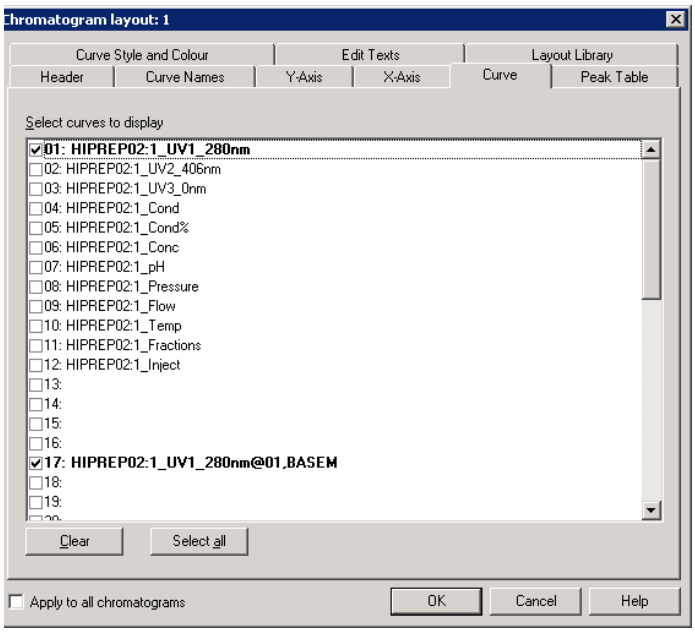


Fig 9-4. Chromatogram Layout dialogue, Curve tab.

The layout of the three views for header, curves and peak table can be modified in the various tabs that are displayed in the **Chromatogram Layout** dialogue. You can work freely in the **Chromatogram Layout** dialogue and all of the configurations are applied when you click on the **OK** button. If instead you want to close the dialogue without applying the changes you have made, click on the **Cancel** button. The main features of the **Chromatogram Layout** dialogue regarding chromatograms are described in the sections below. Features regarding peak tables are described in chapter 10.

9.2.4 Choosing the curve(s) you want to see

In the **Curve** tab of the **Chromatogram Layout** dialogue is a list of all curves contained within the chromatogram, numbered from 01 onwards. Select the curves you want to see in the chromatogram. Click on **OK** to return to the active chromatogram window.

9.2.5 Displaying curve names

By default, names are sequentially built up from three components:

- result name,
- chromatogram name,
- curve name.

For example, a curve with the name 9139401:1_UV1_280, is derived from the result named 9139401. The chromatogram name is a number automatically given during a run, e.g. 1. The curve name corresponds to the curve type, e.g. UV1 for UV detection of an eluted component. If two or more curves of the same type were created within a result file, they will be numbered accordingly, e.g. UV1, UV2 etc. For systems using a variable wavelength detector, the wavelength for the UV curve is also given, e.g. 280.

If you do not want to display the entire names of the curves in both the dialogues and chromatogram windows:

1. Click on the **Curve Names** tab in the **Chromatogram Layout** dialogue.

2. Check the appropriate option boxes for the **Curve name appearance**.

3. Select the appropriate **Curve legend position** option.

It is usually sufficient to select the **Curve Name** option if only one chromatogram is being evaluated. However, confusion may arise when more than one chromatogram is shown, so more complete names may be necessary.

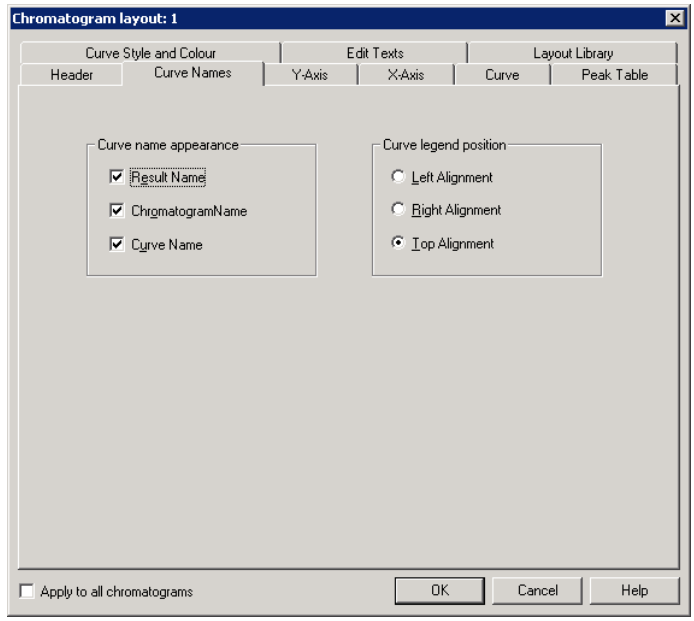


Fig 9-5. Curve Names dialogue.

9.2.6 Changing the colour and style of curves

All curves within a chromatogram are represented by a default colour and line style. Curves imported into the chromatogram or newly created curves are automatically assigned a colour and line style.

To reassign the colour and/or style of a specific curve, do the following:

1. In the **Chromatogram Layout** dialogue, select the **Curve Style and Colour** tab.

2. To change the colour and/or line style of a curve, select the curve of interest from the list.

3. Select the desired colour and/or style.

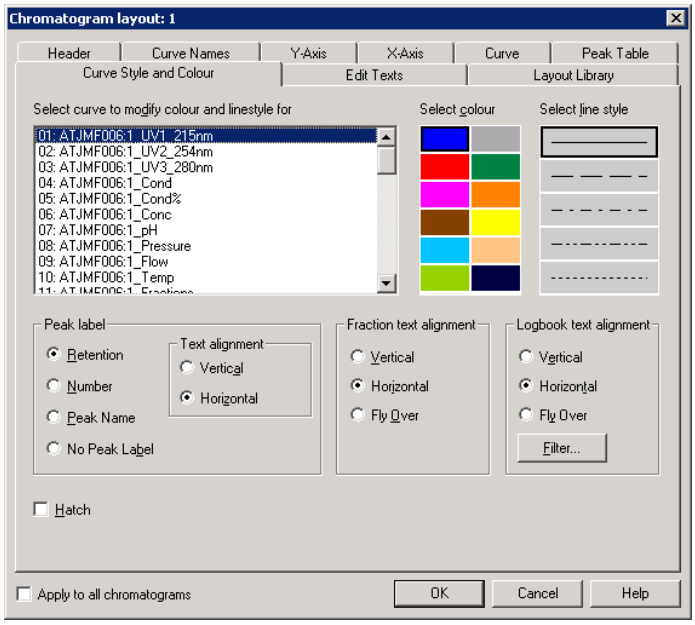


Fig 9-6. Curve Style and Colour dialogue.

9.2.7 Defining and positioning curve text

The **Curve Style and Colour** dialogue also allows for variable forms of peak labelling, as well as alignment of **Fraction** and **Logbook** text.

Peaks may be labelled according to **Retention times** (the default label), by sequential **Number**, or by user-defined **Peak name**. The **Peak label** text can be set to either **Vertical** or **Horizontal**. Checking the **No Peak Label** box removes peak labels.

Both **Fraction text** and **Logbook text** can be set to **Vertical**, **Horizontal** or **Fly Over** alignment by checking the appropriate box. **Fly Over** alignment sets text labels as hidden text, which appear only when the cursor is carefully positioned over a curve line.

Within the **Logbook text alignment** field, clicking on the **Filter** button opens the **Filter Logbook** dialogue. Choose the radio button for the logbook information to be filtered, set the maximum block depth, then click **OK**. To see the mark you must also select the curve (position 22 in the curve list).

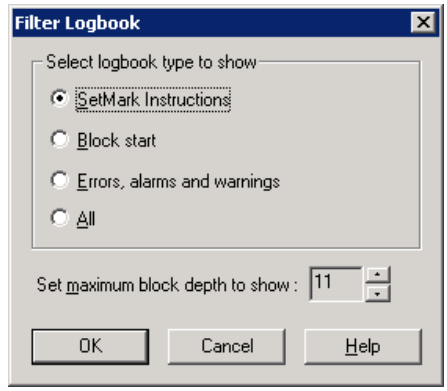


Fig 9-7. Filter Logbook dialogue.

9.2.8 Changing and fixing the axes

By default, the Y-axes are automatically scaled for each curve to show the whole curve. The X-axis automatically scaled to display the run from the point of injection (ml or minutes).

It is possible to 'fix' the minimum and maximum values for the axes of any curve and thereby select a specific part of the curve to be displayed.

Y-Axis

1. Click on the **Y-Axis** tab in the **Chromatogram Layout** dialogue.

2. Select the appropriate curve from the list for which you want to fix the scale. Click on the **Fixed** option.

3. Type in the desired minimum and maximum values for the Y-axis. If you click on **All with this unit**, other curves that have the same Y-axis units as the current scaled curve will be similarly scaled. Click on **OK**.

Note: For pressure curves, Y-Axis units may be changed by clicking on the appropriate **Pressure unit** (MPa, psi, bar). The default **Pressure unit** is **From strategy**, which is the unit defined in the original run strategy.

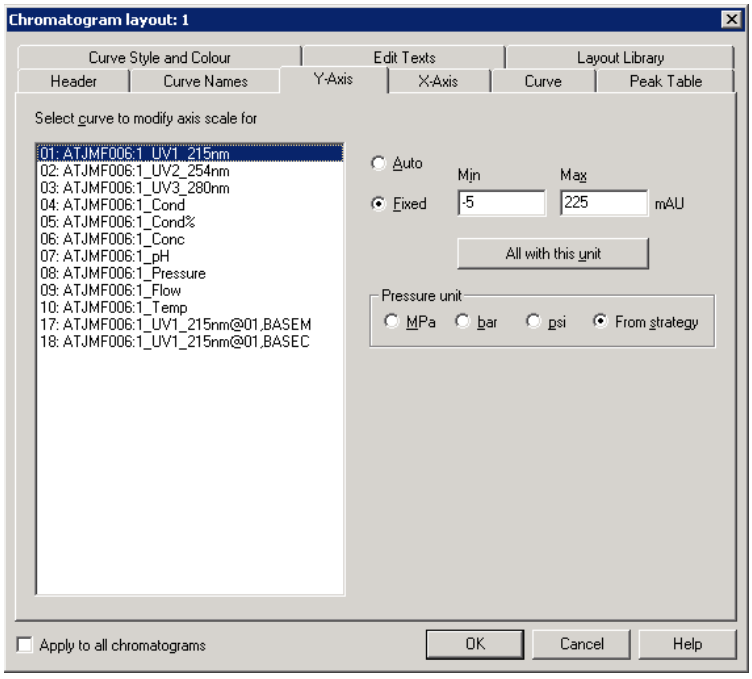


Fig 9-8. Chromatogram Layout dialogue, Y-Axis tab.

Note: **All with this unit** will only be applied to existing curves. It will not be applied to new curves created after this function was last used. New curves are automatically scaled.

X-Axis

1. Click on the **X-Axis** tab in the **Chromatogram Layout** dialogue.

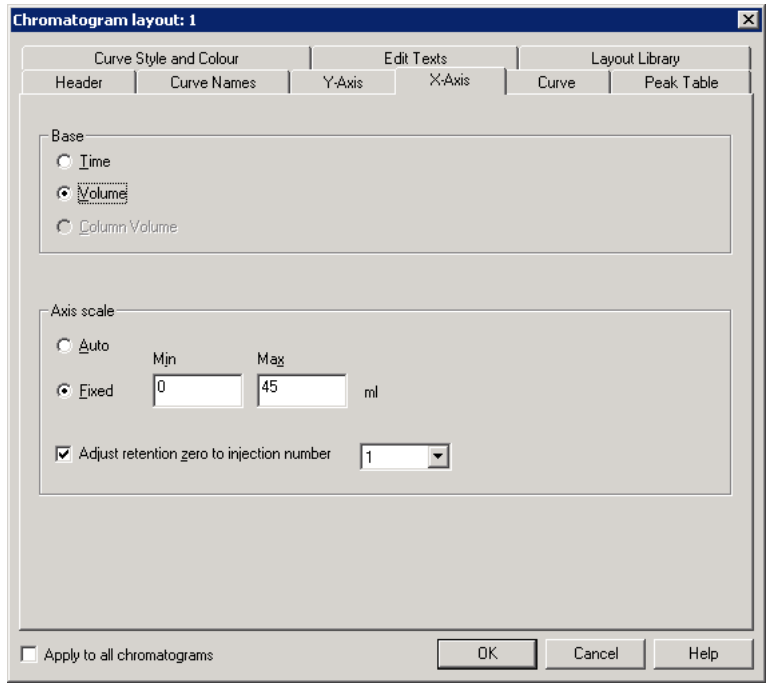


Fig 9-9. Chromatogram Layout dialogue, X-Axis tab.

2. Select the appropriate choice from the **Base** field, either **Time** of retention, **Volume** or **Column volume**.

Note: Some calculated curves, e.g. baselines, exist in only one base and may seem to "disappear" when the base is changed. Curves are collected in time and recalculated for display in volume. Thus, switching the base between **Time** and **Volume** may slightly alter the resolution.

3. To manually set the axis limits, click on the **Axis Scale**, **Fixed** option. Type in the desired minimum and maximum values for the X-axis.

4. The **Adjust retention zero to injection number** checkbox is checked by default. This function sets the time/volume to zero at the injection mark, i.e. when the sample was injected. The time and volume before injection will become negative values. The injection mark to be used as zero is denoted by the number.

9.2.9 Viewing information about the run

You may wish to display header information at the top of a chromatogram detailing the variables, scouting variables, questions and/or notes. Header information cannot be displayed for imported chromatograms.

1. In the **Chromatogram Layout** dialogue, click on the **Header** tab.

2. Check the options to be included in the header of the chromatogram window and click on **OK**. In the chromatogram window, place the cursor at the top of the curve window (just below the toolbar) until the window sizing tool appears, then drag the cursor down to expose the header window.

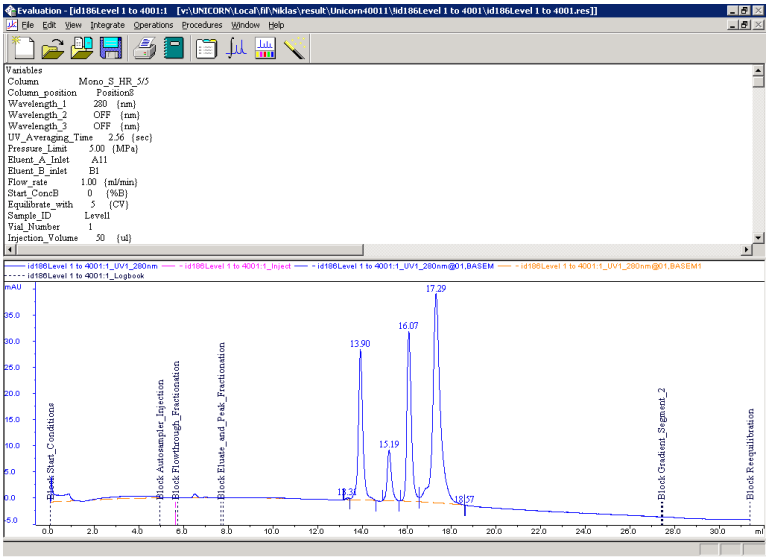


Fig 9-10. Chromatogram with header information displayed.

9.2.10 Saving and applying a layout

All configurations that you make in the **Chromatogram Layout** dialogue can be saved as a layout. It is possible to apply saved layouts to other chromatograms. All saved layouts are user specific.

To save a layout

1. Open the **Chromatogram Layout** dialogue and make the appropriate layout configuration within the various tabs. Note that you can return to the chromatogram window by clicking on

OK to see the applied affects of a given configuration and return again to the **Chromatogram Layout** dialogue to perform further changes.

2. Select the **Layout Library** tab and click on **Save current layout as**.

3. Enter a name for the layout in the displayed dialogue. If you want the current layout to be the new default layout, check the **Save as default** option.

4. Click on **OK** to save the layout. The new name is added to the **Saved layouts** list.

To apply a layout

1. Select the **Layout Library** tab.

2. Select a layout from the **Saved layouts** list and click on the **Apply selected layout** button. The layout is automatically applied to the active chromatogram window. If the same layout is to be applied to all chromatograms on the Evaluation workspace, select (check) the **Apply to all chromatograms** option.

9.2.11 Displaying a hatched background in the chromatogram window

To display a hatched background in the chromatogram window:

1. From the shortcut menu, select **Hatch**. The hatching can be removed by selecting the **Hatch** option again.

When working with more than one chromatogram in the Evaluation window, it is easier to apply hatching by:

1. In the **Chromatogram Layout** dialogue, select the **Curve Style and Colour** tab.

2. Check the **Hatch** option, then the **Apply to all chromatograms** option, then click **OK**. Hatching is now applied to all open chromatograms.

To remove the hatching, uncheck the **Hatch** option.

9.3 Other presentation possibilities

The Evaluation module allows you to perform operations on the curves to optimise the presentation.

9.3.1 Changing the size of fraction marks

The following steps outline the procedure for changing the size of the Fraction marks:

1. Shutdown the UNICORN software.

2. Open the file in Notepad and locate the following line:

   ```
   EVAL FracSize - 10.0
   ```

3. If the line is not present, add it under the line:

   ```
   EVAL Print Margin Bottom 5
   ```

4. Find a suitable value for the fraction size by arbitrarily increasing or decreasing the value - 10.0. Negative values set size in % of window height, while positive values set size in character heights. If the line "EVAL FracSize" is not present, the default size will be 50% of window height.

5. Save the file.

Note: Do not make any changes in the UNICORN.INI file between the lines "BEGIN" and "END" as this may severely affect the functionality of UNICORN.

9.3.2 Showing part of a curve

This section deals with the selection of just part of a curve for purposes of closer examination of details and for presentation. This can be done in three different ways:

- magnification using the zoom function
- fixing the axes
- cutting the curves

The zoom function

In the active chromatogram window, it is possible to zoom in on a designated area of the chromatogram. This is the easiest and quickest way to enlarge different parts of a curve.

1. Place the mouse pointer in any corner of the intended area to be magnified.

2. Press and hold the left mouse button. A magnifying-glass icon will will be added to the mouse pointer arrow on the screen.

3. Drag out a box from the point of origin to cover the area to be magnified. Release the mouse button.

   The selected region is now displayed in the entire chromatogram window, together with appropriate scales for the Y- and X-axes.

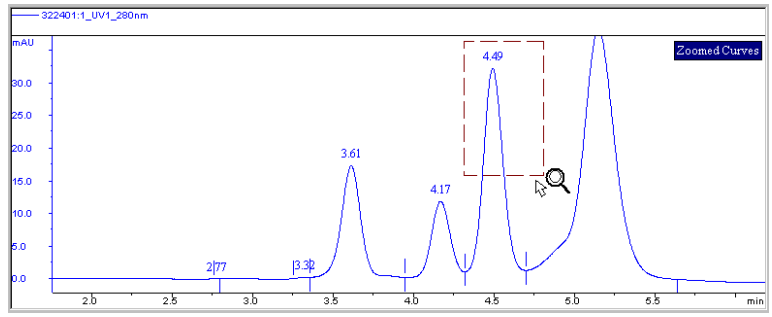


Fig 9-11. Illustration of the chromatogram Zoom function.

4. To move around in the chromatogram at the current zoom scale, use the cursor-arrow keys on the keyboard.

5. To undo the last zoom step, select **Undo zoom** from the shortcut menu. To reset all zoom steps at once, i.e. no zoom applied, select **Reset zoom** from the shortcut menu.

Alternatively:

Use the <Page Down> and <Page Up> keys to zoom in and zoom out, respectively, on the whole chromatogram.

Fixed scale axes

Another way to display only part of a curve is to choose, or 'fix', the minimum and maximum values of the Y- and/or X-axes in the **Chromatogram Layout** dialogue (see "Changing and fixing the axes" on page 192).

Cutting curves

The cut curve function allows a region of the curve between two values on the X-axis to be cut and stored as a new curve. This is done in the following way:

1. Select **Operations:Cut curve**.

2. In the displayed dialogue select the curve(s) to be operated on. Click on **OK**.

3. The selected curves will now be shown in a new window which also contains two vertical cursor lines. To facilitate the cutting process, it is possible to use the zoom function within the window.

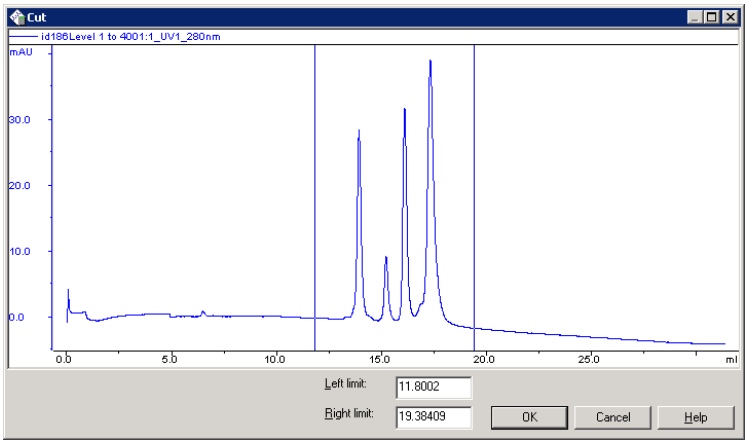


Fig 9-12. Cut window.

To select the region to be cut, either:

- drag the two cursor lines to define the left and right limits of the cut area, or,
- type the desired left and right limit values in the boxes marked **Left limit** and **Right limit**.

Note: The areas outside of the **Left limit** and **Right limit** will not be saved in the newly created cut curve. Thus, the X-axis of the new saved curve will not begin at zero unless designated as one of the limits. The original curve is not changed.

4. Click on **OK**. A new dialogue is displayed. Select whether to save the new cut curve in the **Source chromatogram**, i.e. the current active chromatogram, or in a **New chromatogram**. If you select the latter option, you can change the name of the chromatogram. Click on **OK**.

5. If the destination of the cut curve was the source chromatogram, the cut curve is automatically displayed in the source chromatogram. If the destination of the cut curve was a new chromatogram, this will be represented as a new, open chromatogram window.

9.3.3 Reducing noise and removing ghost peaks

Sometimes the chromatograms may contain curves with a noisy baseline. The noise can be caused by several things e.g. a dirty flow cell, air bubbles, electrical noise, dirty buffers etc. The amount of noise can usually be reduced by taking proper precautions, e.g. filtration of buffers and instrument maintenance.

Smoothing a curve

The smoothing function allows background noise to be reduced or removed from any selected curve. The type of smoothing function you should choose depends upon the type of noise encountered.

1. Select **Operations:Smooth**.

2. Select the source curve to be smoothed and its target destination. By default, smoothed curves are given the suffix, **SMTH**.

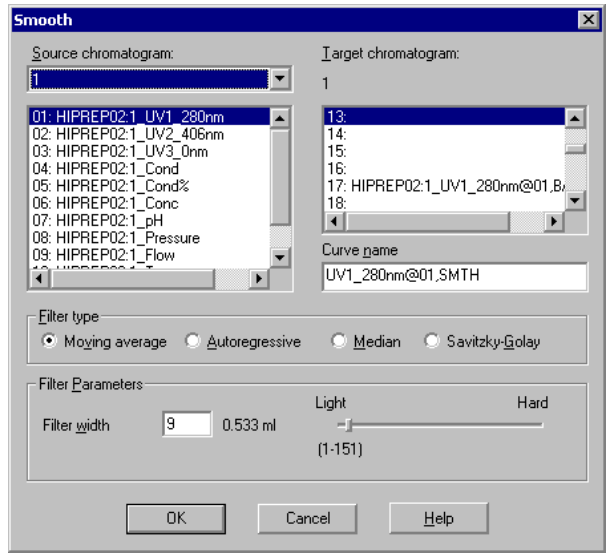


Fig 9-13. Smooth dialogue.

3. Select the **Filter type** to be applied in the smoothing operation. This selection can be based on the following criteria:

   - Choose **Moving average** if you have noise along most of the curve.
     Smoothing with this filter affects peak height but not retention. There is little effect on peak area.

- Choose **Autoregressive** if you have periodic noise along the whole curve.
  Smoothing with this filter will affect peak height and retention, although has little affect on peak area.
- Choose **Median** if there is only one or a few noise spikes, e.g. caused by air bubbles, or if the noise is confined to only a small part of the curve.
  Smoothing with this filter may give flattened peaks and affect peak areas slightly but will not affect the retention.
- Choose **Savitzky-Golay** algorithm to calculate the smoothing and differntiation of data by a least squares technique.

4. Select an appropriate smoothing parameter value from **Light** to **Hard** for the selected filter. This can be done using the Filter width slider, or you can manually insert a value in the **Filter width** text field. The smoothing effect increases with increasing parameter values.

   Smoothing is always a compromise between noise removal and preservation of peak shape. The easiest way to find the optimum smoothing effect is to start with a low parameter value, e.g. the default value, and increase it until the best result is achieved. A useful strategy is to increment the parameter value by the default value for each try.

5. Click on **OK**.

The formulae for the filters are described in see "Smoothing algorithms" on page 369.

9.3.4 Subtracting a blank run curve

This is a frequently used function in presentations, especially if the curves have a drifting baseline or "ghost" peaks.

Note: If the ghost peaks come from impurities in the eluents, all equilibration of the columns should be the same from run to run. If, for example, the equilibration volume with buffer A is larger before a blank run curve than before a separation, your ghost peaks might be higher in the blank run curve.

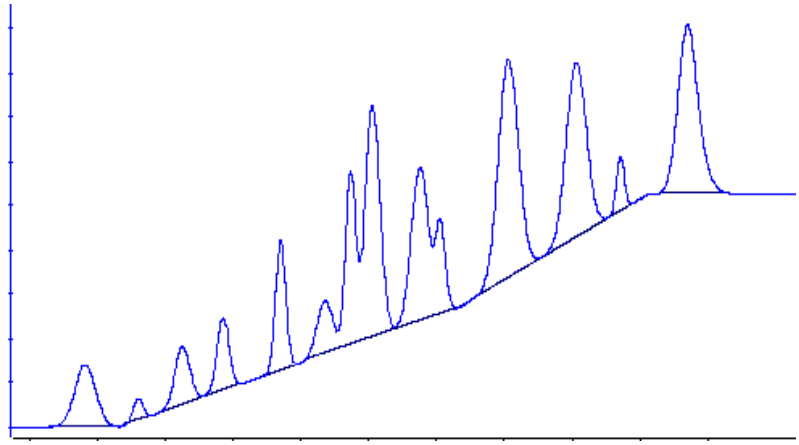


Fig 9-14. UV curve with baseline prior to subtraction of the baseline.

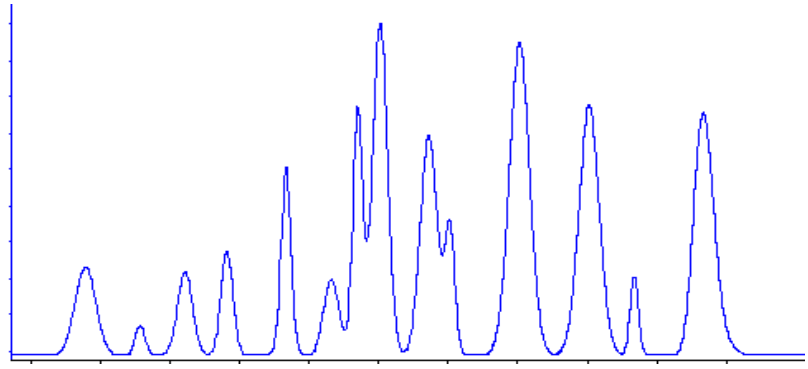


Fig 9-15. UV curve after subtraction of the baseline.

Alternative A: Importing a blank run curve

If a blank run curve was done, this may have been stored in another result file. To access the blank run curve:

1. Ensure that the destination chromatogram has been opened and is the active window on the workspace.

2. Select **File:Open** and then select **Curves** from the menu cascade. The **Open Curves** dialogue is displayed.

3. Locate and double click on the result file containing the blank run curve. The curves in the first chromatogram are displayed.

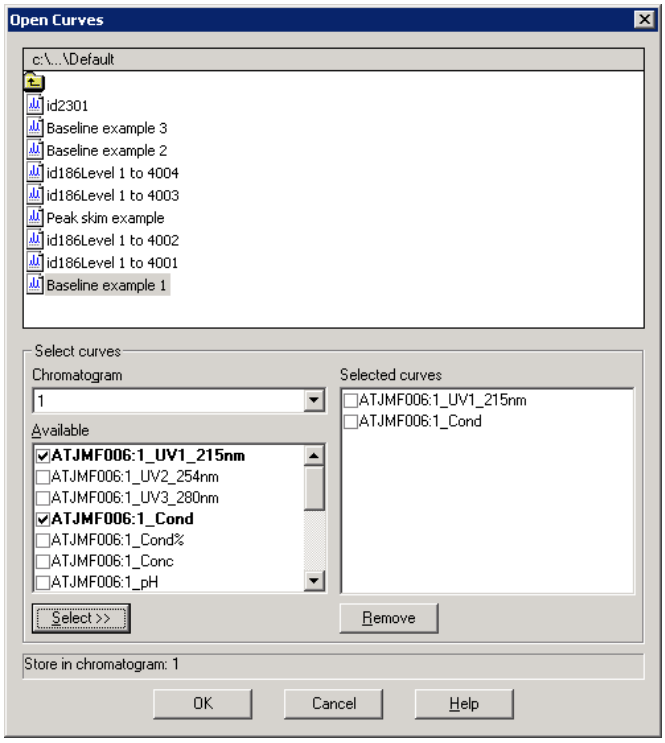


Fig 9-16. Open Curves dialogue with a result file selected.

4. Check the curve corresponding to the blank run curve and click on the **Select** button. The selected curve will now be displayed in the **Selected curves** list.

   To remove a curve from the list, check it and click on **Remove**.

   If there is more than one chromatogram in the result file and the blank run curve resides in another chromatogram, select the appropriate chromatogram from the drop-down list. The curves for that chromatogram are displayed from which you should make the appropriate selection.

5. To import the curve click on **OK**.

Note: For more detailed information about how to import curves, chromatograms and other results, see "Comparing different runs" on page 206.

Alternative B: Calculating a baseline

If there is no blank run curve, you can instead create a baseline with **Integrate:Calculate baseline** (see "Baseline calculation for integration" on page 259).

Subtracting the blank run curve

Select **Operations:Subtract** to subtract the blank run curve or the baseline away from the sample curve. Click on **OK**. All resulting curves from the subtract operation receive the SUB suffix.

9.3.5 Adding curves

In some runs, several sequential chromatograms may have been created, for example, when the instruction **New chromatogram** has been used in the method, thus creating different chromatograms during the run. In order to view and evaluate the resultant curve of all the chromatogram parts, the curves must be added together. The common situation is when you have a number of chromatograms within the same result file and you want to add the curves. In some circumstances, curves may need to be imported from other result files.

To add curves:

1. Select and view the first chromatogram in the sequence.
2. Select **Operations:Add**. Add the first curve in the sequence to the second curve in the sequence from the appropriate chromatograms.
3. Add the result of the previous step to the next curve in the sequence.
4. Repeat this process until all curves have been added together. The final curve should be the cumulative curve for the whole run. All curves created using the **Add** operation receive the ADD suffix.

9.3.6 Entering text in the chromatogram

Basic annotations can be added to the chromatogram.

1. Place the mouse pointer in the curves view of the chromatogram window and select **Add text** from the shortcut menu. Alternatively select the **Edit:Text:Add** command. The mouse pointer is replaced with an **abc** pointer.
2. Position the pointer where you want to insert text in the chromatogram and click the left mouse button once.
3. Type in the desired text and then click outside the text box to set the text.

If you want to edit, change fonts, move, re-orient or delete an inserted text, either:

- Place the cursor anywhere over the active curve and select **Edit Text Mode** from the shortcut menu, highlight the text, then choose the desired function from the shortcut menu.

or

1. Open the **Chromatogram Layout** dialogue and select the **Edit Texts** tab. Alternatively, select the **Edit:Text:Edit** command and the **Edit Texts** tab is displayed automatically.

2. Select the specific text that you want to edit and make the appropriate changes in the **Selected text** field. Click on **Change text** or **Delete text**.

3. To alter the font or text orientation, select the specific text that you want to modify and click on **Font** or **Set Orientation**. Make the desired changes in the resulting dialogues.

4. Click on **OK** to close the dialogue and apply the changes.

9.3.7 Pooling fractions

During a separation, fractions are sequentially collected, with each fraction containing a set volume of sample. Each fraction is numbered according to its order in the sequence and this information is saved as a curve under the name **Fractions**. Selection of this curve in the **Chromatogram Layout** dialogue will display the precise contents of each fraction relative to the information displayed on the UV detection curve.

Since the peak of interest may not always be collected in one fraction, it may be desirable to pool some fractions.

To pool the fractions:

1. Select **Operations:Pool fractions**. The **Frac curve** is automatically displayed for the active chromatogram. The chromatogram selection can, of course, be changed.

2. In the **Fraction no.** field, check the fractions to be pooled.

3. Select the destination for the new curve. The default name of the new curve has POOL as its suffix.

3. Click on **OK**.

The active chromatogram will now display both the original and pooled fraction curves, so uncheck the original fraction curve in the **Chromatogram Layout** dialogue.

9.3.8 Matching protein activity to a curve

The Evaluation operations allow you to combine data from the results of protein activity assays, for example ELISA, with the data contained on the UV curve. A presentation comparing the activity curve with the UV curve can then be made.

1. Select **Operations:Activity histogram**. A dialogue is displayed with the fraction curve for the specific chromatogram already selected. Change the source and target chromatogram selection if desired.

2. All the component fractions of the fractions curve are listed. For each fraction you can type in the corresponding activity value. When all values have been entered, click on **OK**.

9.3.9 Renaming chromatograms, curves and peak tables

Sometimes, it may be desirable to change the name of a chromatogram, curve or peak table. To do this, close the **Chromatogram Layout** dialogue and then:

1. Select **Edit:Rename** and the relevant menu cascade option **Chromatogram**, **Curve** or **Peak Table**.

2. Select the appropriate object in the displayed dialogue and type in the new name. Click on **OK**. The new name will replace the old one rather than creating a new curve or chromatogram.

Note: The original raw data curves cannot be renamed and are not therefore given as options.

9.4 Comparing different runs

The previous sections dealt with the manipulation of single curves within a chromatogram. The following sections describe how to make comparisons between and how to present two or more curves or chromatograms from different runs.

It is possible to:

- compare curve parameters among curves from different runs
- view several chromatograms at the same time
- overlay curves from different runs in one chromatogram

- stack curves from different runs in one chromatogram
- stretch curves to make comparisons easier
- create mirror images

9.4.1 Multifile Peak Compare

9.4.2 Wizard set-up

To open **Multifile Peak Compare**, choose **File:Multifile Peak Compare:Start Wizard** or click on the **Multifile Peak Compare toolbar icon**. This will prompt the **Multifile Peak Compare** Wizard entry dialogue. Click on the **Next** button to display the **Operation** dialogue.

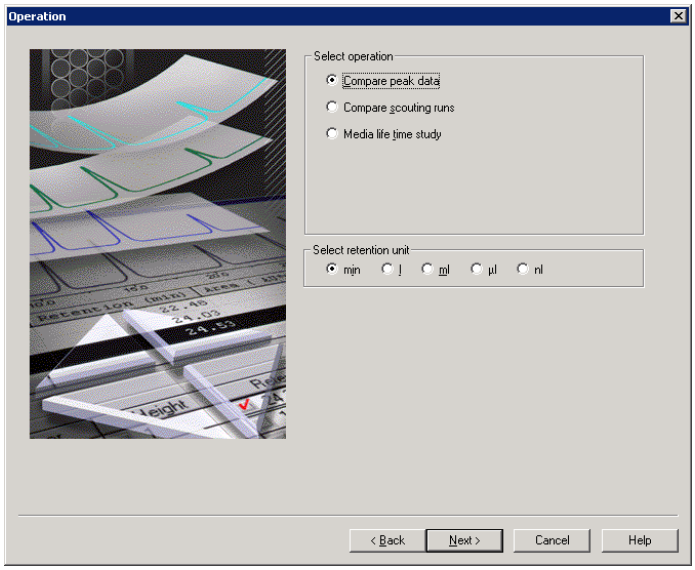


Fig 9-17. Operation dialogue within Multifile Peak Compare.

Select from the available operations, choose a retention unit, and click on **Next**. This will prompt the **Data Selection** dialogue.

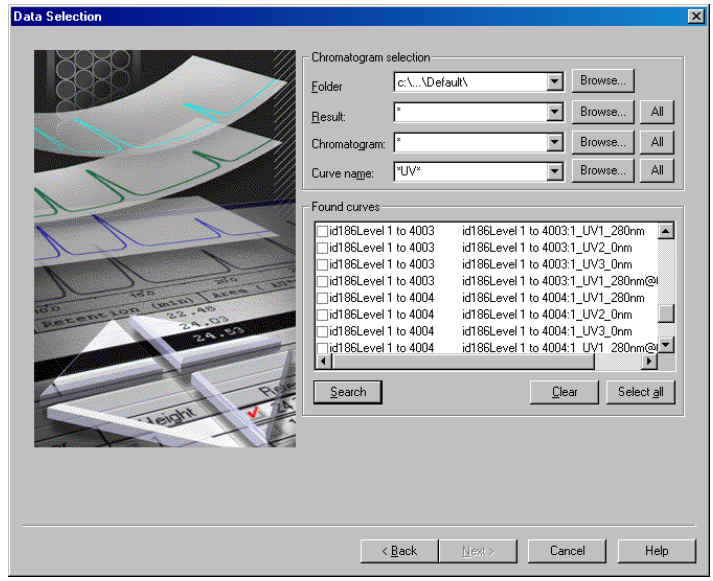


Fig 9-18. Data Selection dialogue.

1. Use the **Chromatogram selection** drop-down menus to identify the results files for comparison, then click on the **Search** button in the **Found curves** field. A list of all curves that matched the search criteria is displayed in the **Found curves** field.
2. Use the check boxes (or click on the **Select All** button) to identify the desired curves within the **Found curves** field, then click on **Next**.

Note: If any of the chosen curves have not been integrated, the following dialogue is prompted:

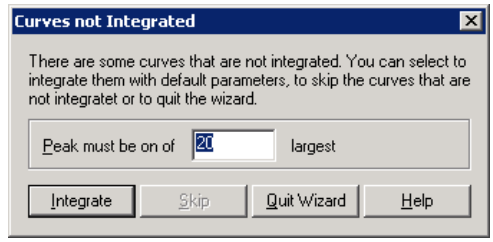

Curves not Integrated
There are some curves that are not integrated. You can select to integrate them with default parameters, to skip the curves that are not integratet or to quit the wizard.
Peak must be on of
20
largest
Integrate
Skip
Quit Wizard
Help

Fig 9-19. Curves not Integrated dialogue.

If desired, change the default value for peak number selection filter, then click on **Integrate**. The **Peak Selection** dialogue is prompted.

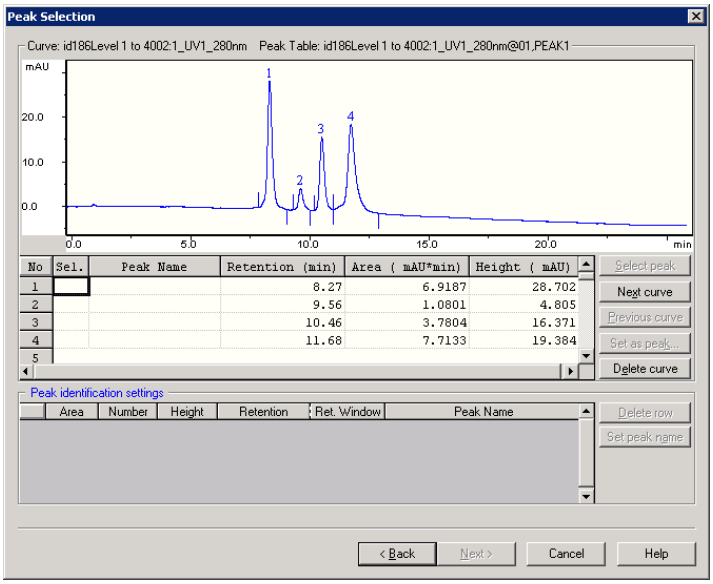


Fig 9-20. Peak Selection dialogue.

The **Peak Selection** dialogue displays the integrated peak for the first of the chosen curves, the associated peak table, as well as a **Peak identification settings** table that is used to identify which of the peak parameters will be used in the comparison. Follow the steps below to complete the dialogue:

1. In the curve window, choose the desired peak either by double clicking directly on the peak, or by clicking once and then clicking on the **Select peak** button. The peak is assigned a letter designation (A, B, C....) and the peak parameters are displayed in the **Peak identification settings** table.

Note: If the peaks in the curve window do not appear to be integrated properly (e.g., "ghost" peaks are labelled), quit the Wizard and perform a **Peak Integration** on the selected curve. Check to see that the resulting curve has been properly integrated, then repeat the **Multifile Peak Compare** Wizard operation.

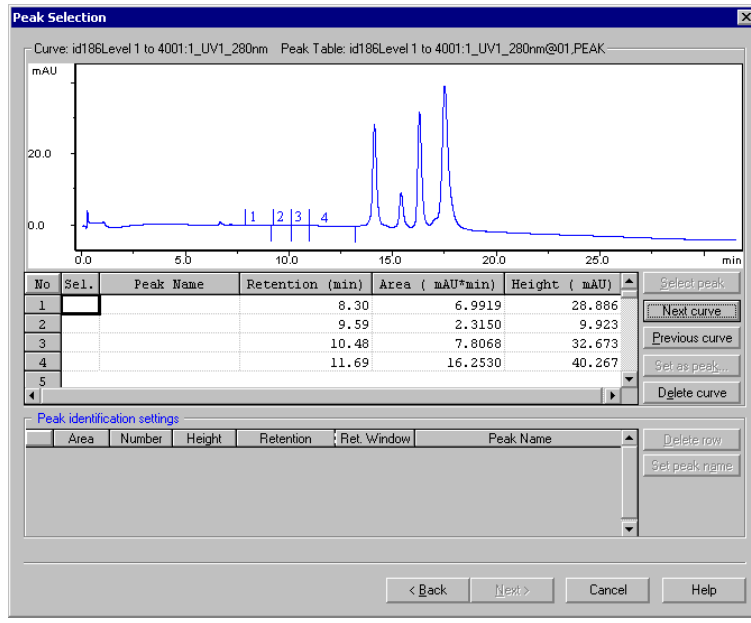


Fig 9-21. Peak Selection dialogue showing “ghost” peaks in the curve window.

2. Set the desired peak identification criterion by clicking on the desired parameter in the **Peak identification settings** table. For example, if you have selected the highest peak in the curve and want to compare the highest peak among all curves, check the **Height** check box.

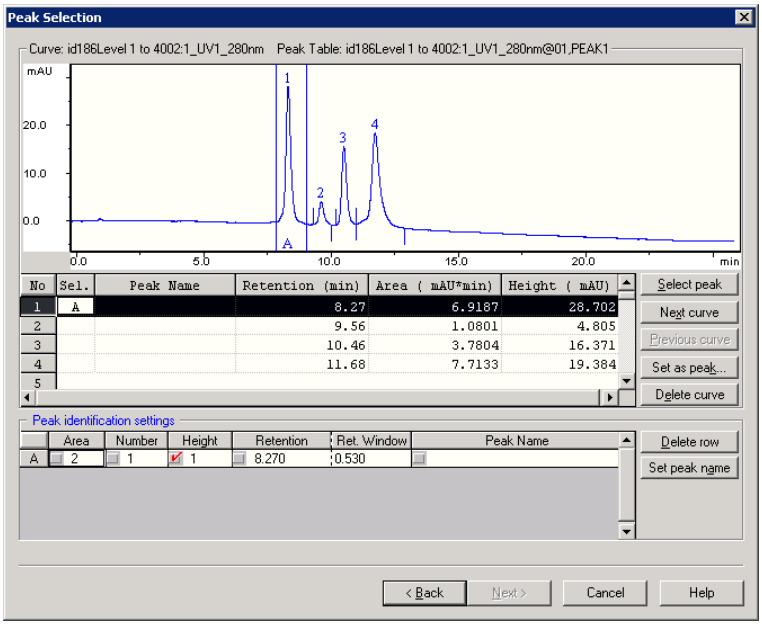


Fig 9-22. Peak Selection dialogue showing selection of initial (A) peak.

3. Use the **Next curve** and **Previous curve** buttons to navigate forward and backward among your selected curves, manually checking the selections made by the software.

4. If desired, assign a name to a chosen peak by highlighting the appropriate row and then clicking on the **Set peak name** button. This can be useful when comparing multiple peak parameters, and you wish to have peak names other than Peak A, Peak B, etc....

5. Follow steps 1-4 for any other desired peaks in the current curve, or navigate to other curves and follow steps 1-4 above.

6. To change the designation of a peak, either:

   - click on the desired peak in the curves window, click on the **Set as peak** button and choose **None**. Note that you must also select the same peak in the **Peak identification settings** table, and click on **Delete row.**

   or

- select a new peak, click on the **Set as peak** button, and click on the letter of the old peak (the one to be replaced) in the list.

7. If the current curve does not prove useful for your comparison, use the **Delete curve** button to delete it from the comparison. Also, at any point you can use the **Back** button to navigate back to the **Data Selection** dialogue and add any new curves to your comparison.

8. In the **Peak identification settings** field, each row identifies a peak parameter to be compared among all peaks. Use the **Delete row** button to remove the (selected) row from the field (if you select **Delete row** without first highlighting a row, the first row (A) is deleted by default).

9. When all peak selections and identification settings are complete, click on the **Next** button to proceed to the **Peak Data selection** dialogue.

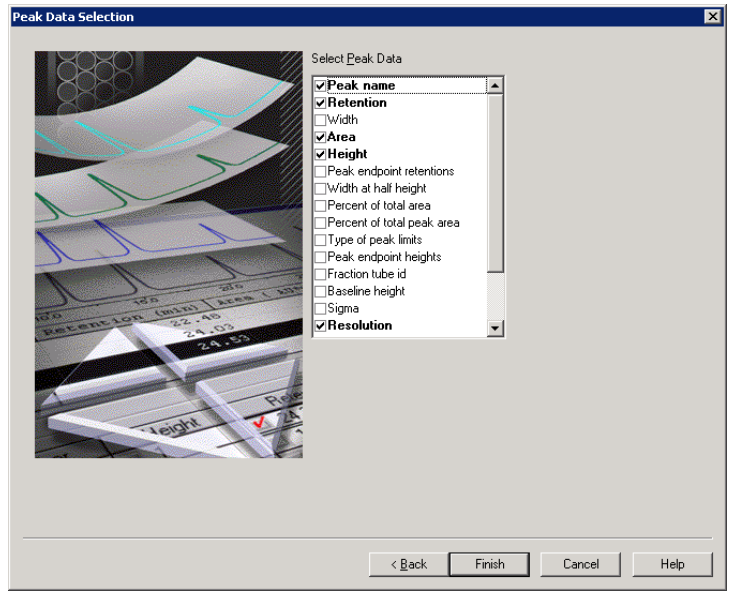


Fig 9-23. Peak Data Selection dialogue.

Use the **Peak Data Selection** dialogue to identify any peak characteristics to be included in your comparisons. This is the last point from which you can use the **Back** button to navigate backwards in the **Wizard**. Click on the **Finish** button to prompt the **Data View** dialogue or the **2-D Data View** dialogue (if **Media life time study** was chosen).

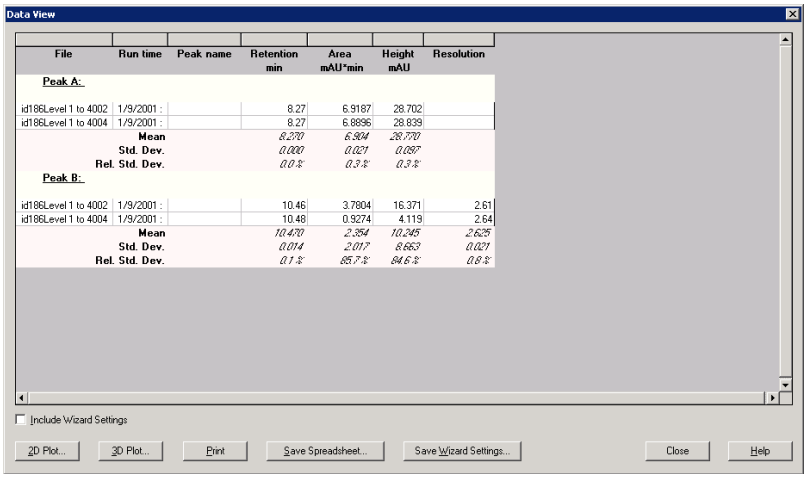

Data View
File
Run time
Peak name
Retention min
Area mAU*min
Height mAU
Resolution
Peak A:
id186Level 1 to 4002 1/9/2001 : 8.27 6.9187 28.702
id186Level 1 to 4004 1/9/2001 : 8.27 6.8896 28.839
Mean 8.270 6.904 28.770
Std. Dev. 0.000 0.021 0.097
Rel. Std. Dev. 0.0 % 0.3 % 0.3 %
Peak B:
id186Level 1 to 4002 1/9/2001 : 10.46 3.7804 16.371 2.61
id186Level 1 to 4004 1/9/2001 : 10.48 0.9274 4.119 2.64
Mean 10.470 2.354 10.245 2.625
Std. Dev. 0.014 2.017 8.663 0.021
Rel. Std. Dev. 0.1 % 85.7 % 84.6 % 0.8 %
Include Wizard Settings
2D Plot...
3D Plot...
Print
Save Spreadsheet...
Save Wizard Settings...
Close
Help

Fig 9-24. Data View dialogue.

The **Data View** dialogue presents a comparison of the chosen data for the designated peak comparisons. Use the **Print** button to print out the spreadsheet. The **Save Spreadsheet** button allows you to save the data in Excel (.xls), tabbed text (.txt), or FarPoint spread (.ss3) formats.

To view the data in 2-dimensional plot, click on the **2D Plot...** button.

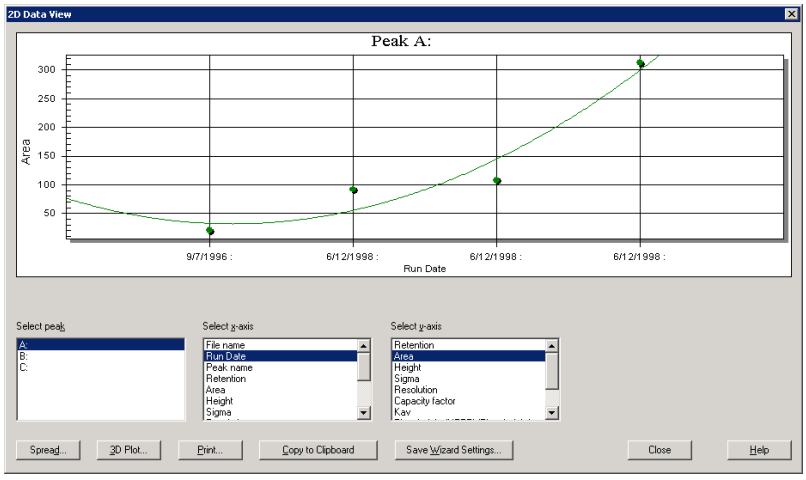


Fig 9-25. 2D Data View dialogue.

Use the **Select peak, Select y-axis** and **Select x-axis** lists to select different peaks and different axes.

A wide array of plot presentation options can be found under the shortcut menu, including a **Customization Dialogue**, allowing further customization of the graph.

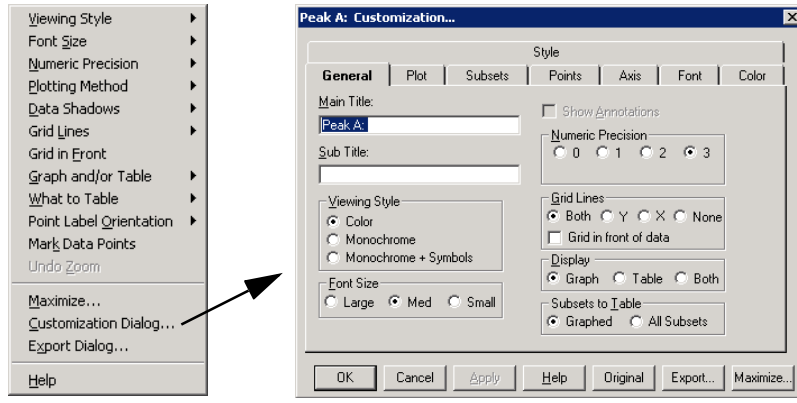


Fig 9-26. Shortcut menu and Customization dialogue within 2D view.

To export the view, select **Export Dialogue** from the shortcut menu, or click on the **Export...** button from within the **Customization** dialogue.

To close the **2D Data View** dialogue, click on the **Close** button. This will end your **Multifile Peak Compare** session. Alternatively, click on **Spread...** to return to the **Data View** dialogue or **3D Plot...** to open the **3D Data View** dialogue.

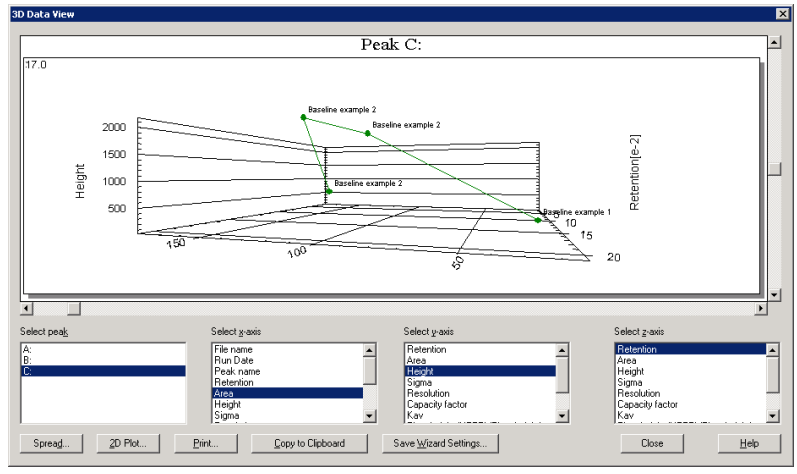


Fig 9-27. 3D Data View dialogue.

The **3D Data View** dialogue is similar to the 2D dialogue, with the addition of a list for altering the variable on the Z-axis, and a different shortcut menu that allows the figure to be viewed by animated rotation.

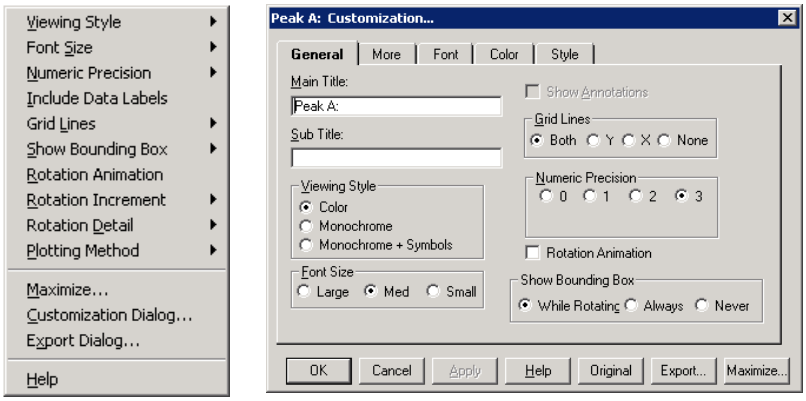


Fig 9-28. Shortcut menu and Customization dialogue within 3D view.

When viewing results in 2D or 3D views, click on **Copy to Clipboard** to copy and store the figure for transfer to external programs.

To save the current Wizard settings for future use, click on the **Save Wizard Settings...** button. Give the Wizard settings a name and if the settings are to be used by all users on the system, check the **Global wizard settings** check box. Click on **OK**.The **Global wizard settings** check box can also be used to toggle between lists of stored global and user settings.

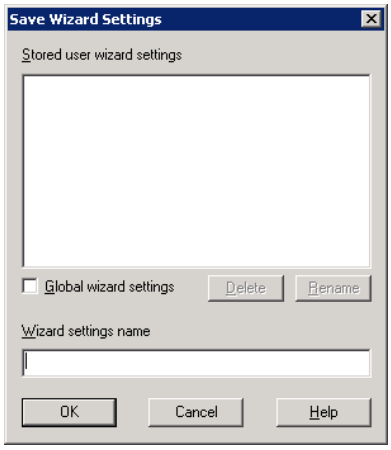


Fig 9-29. Save Wizard Settings dialogue.

From the **Data View** dialogue, click on **OK** to leave the **Multifile Peak Compare** Wizard.

9.4.3 Comparing chromatograms from different runs

To import chromatograms from other result files into an already opened result file, two functions can be used, namely **File:Open to compare** or **File:Open**. The former option is most useful in searching for many chromatograms in a specific folder based on defined selection criteria. The latter option is best used to import any individual chromatograms from result files in different folders. The imported chromatograms are sequentially numbered (11, 12, 13, etc.) for identification purposes. Up to 10 chromatograms can be made available at the same time on the evaluation workspace.

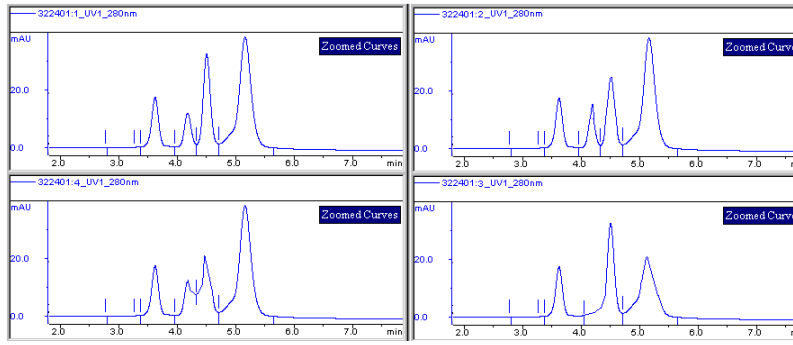


Fig 9-30. Windows:Tile function to display many chromatograms.

Alternative A: Import chromatograms using Open to compare

This method is useful, for example, when importing chromatograms from all files of a scouting folder.

1. Click on **File:Open to compare:Chromatograms**. The **Open Chromatograms to Compare** dialogue is displayed.

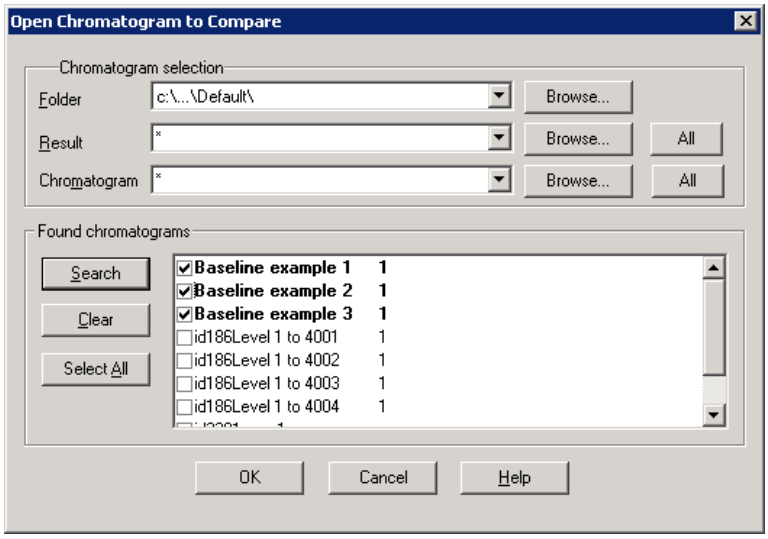


Fig 9-31. Open Chromatograms to Compare dialogue.

2. The search will take place in the displayed folder only. To select another folder, click on the **Browse** button and open the desired folder.

3. The search for chromatograms will take place in all result files within the selected folder as denoted by the asterisk '*****'. You can instead select a specific result file using the **Browse** function. Moreover, you can use wildcard characters to search within result files with a specific name profile.

   You can use standard wildcard characters in the file name specification (* stands for any number of characters and ? for any single character). For example:

iex	will search files named iex
iex*	will search all files with names beginning iex
*iex	will search all files with names ending iex
?iex	will search only 4-character names ending in iex

   User-entered search filters (to a maximum of 10) will be saved in the drop-down menus for both **Result** and **Chromatogram**

selections. More than one string can be used as a search delimiter (by inserting a ';' between strings) and search filters are automatically saved and stored within user profiles.

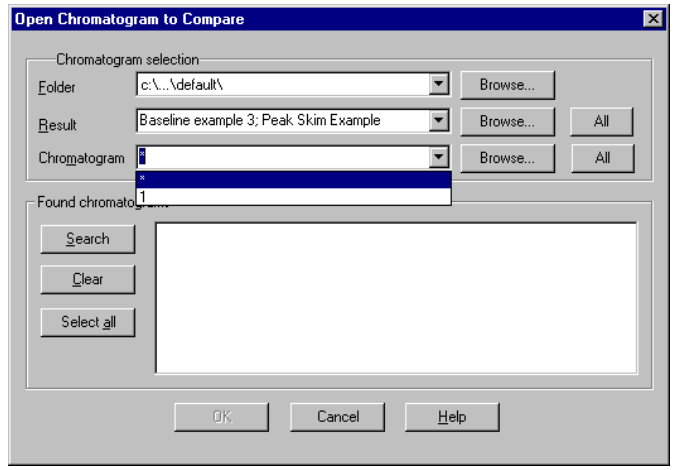


Fig 9-32. Open Chromatogram to Compare dialogue, showing search delimiters.

To return to the default setting to search in all result files, click on **All**.

4. Click on the **Search** button and a list of chromatograms will be displayed based on the designated search criteria. A new search can be performed with new search criteria without erasing the first found chromatograms from the list.

5. Check the chromatograms that you want to import. If you click on the **Select All** button, all of the displayed chromatograms are selected for importing. If you want to clear the list of displayed chromatograms, click on **Clear**.

6. Click on **OK** and all selected chromatograms are shown on the Evaluation workspace.

Alternative B: Importing using Open

1. Select **File:Open:Chromatogram**.

2. Select the desired result file by double clicking on it, and all of the chromatograms contained within will be displayed. Normally it is only one chromatogram and is named "1".

3. Check the chromatogram(s) of interest and press the **Select** button. Selected chromatograms are added to the **Selected chromatograms** list. Chromatograms can be deselected by using the **Remove** command button.

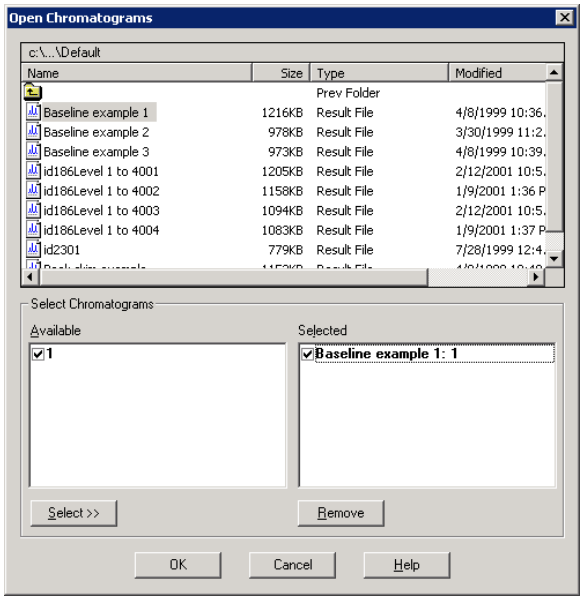


Fig 9-33. Open Chromatograms dialogue.

4. Repeat steps 2-3 for chromatograms in other result files.

5. Click on **OK**.

Viewing all chromatograms

1. Simultaneously display the chromatograms by selecting **Window:Tile** or layer them by selecting **Window:Cascade**.

2. Chromatogram windows can be individually sized and the presentation of the curves changed.

3. If you want to have the same scale on all of the chromatograms, open the **Chromatogram Layout** dialogue for any chromatogram, make the changes and select (check) the **Apply to all chromatograms** option.

Note: Imported chromatograms cannot be shown with column volume as the X-axis base.

9.4.4 Comparing curves

Curves from different runs can be imported or copied into one chromatogram for comparison.

Alternative A: Importing curves using Open to compare

Result files contained in the same folder can be automatically searched to locate all curves of a specified type, for example, all UV curves. This is especially useful for comparison of curves from scouting runs. Moreover, the imported curves can be automatically overlaid, stacked or be presented as mirror images.

1. Select **File:Open to compare:Curves** or click on the **Open curves to compare** toolbar button. Select the search criteria for the folder, result, chromatogram and/or curve name using the respective **Browse** command buttons.

   Wildcard characters, * and ?, can also be used to further specify the search parameters (see "Alternative A: Import chromatograms using Open to compare" on page 216).

   User-entered search filters (to a maximum of 10) will be saved in the drop-down menus for both **Result** and **Chromatogram** selections. More than one string can be used (by inserting a ';' between strings) as a search delimiter and search filters are automatically saved and stored within user profiles.

   The UV curves are identified with number and sometimes wavelength. For example, UV1_280, UV2_280 and UV1_254 are all different curves. To search for all UV curves, enter *UV* in the **Curve name** text field.

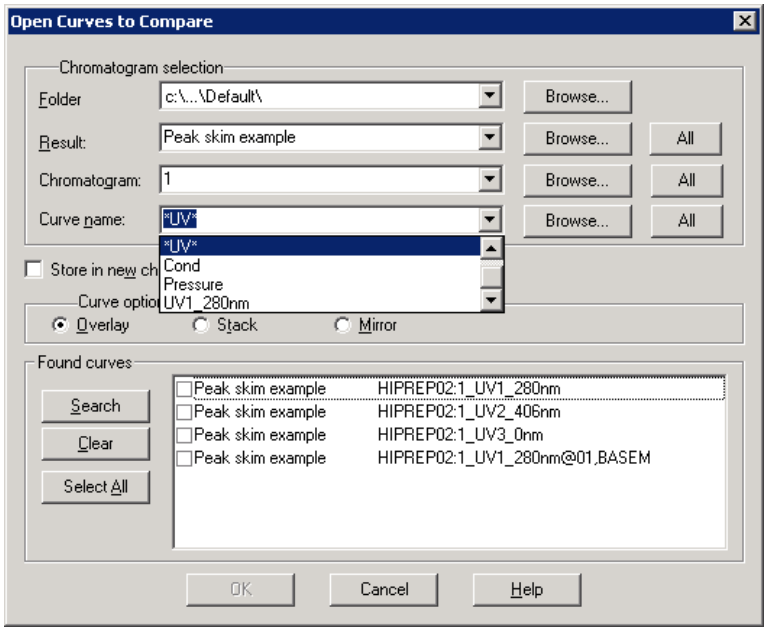


Fig 9-34. Open Curves to Compare dialogue.

2. Click on **Search** and a list of found curves will be displayed based on the designated search criteria. A new search can be performed with new search criteria without erasing curves located in the previous search.

3. Select the curves that you want to be imported. Click on **Select All** if you want to import all of the curves.

4. You can import the curves into a new chromatogram by selecting (checking) the **Store in new chromatogram** option. This is recommended to keep the source chromatogram free of too many additional curves.

5. Select how the imported curves will be displayed by clicking on one of the buttons; **Overlay**, **Stack** or **Mirror**.

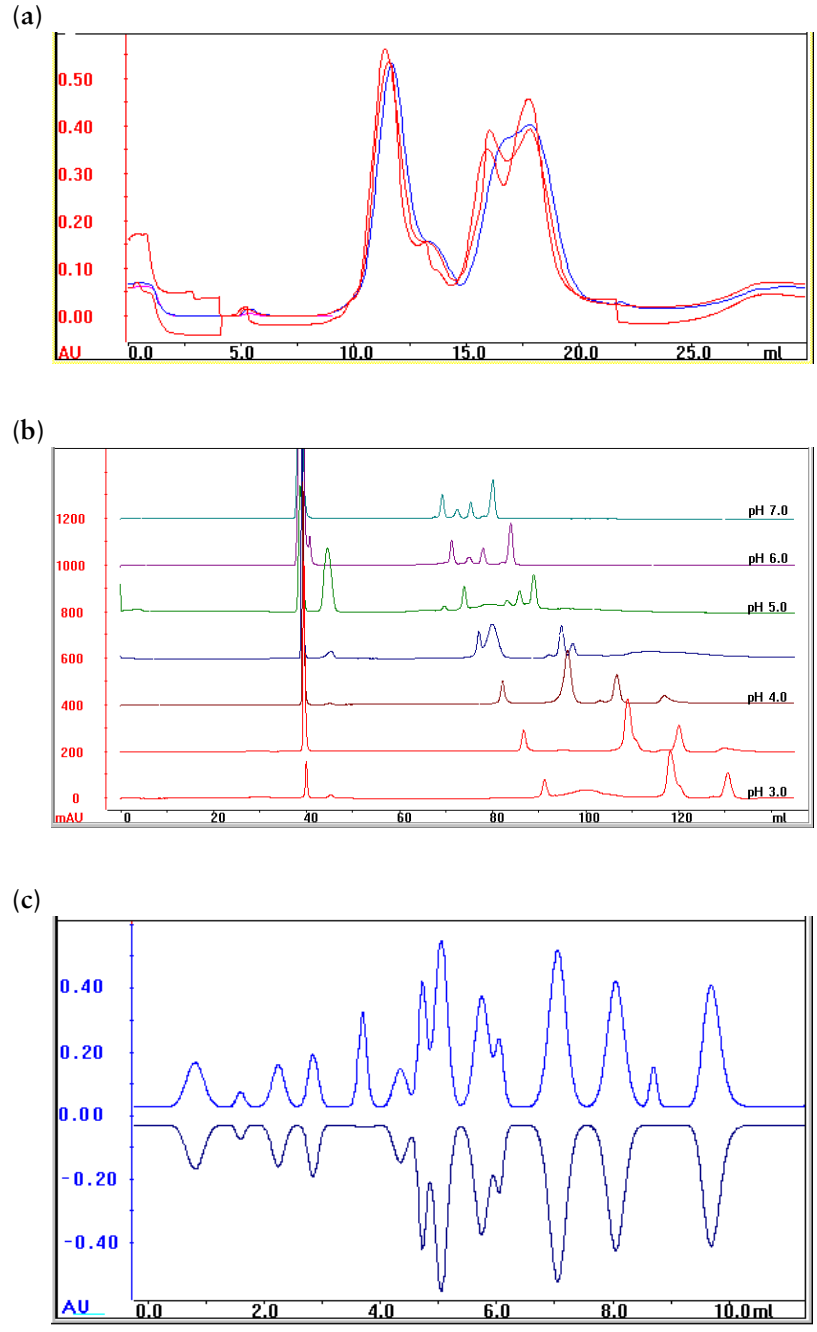


Fig 9-35. Different presentation options for comparison of imported curves; (a) overlaid curves, (b) stacked curves, (c) mirrored curves.

Overlay This presents the imported curves overlaid one on another.

Stack This presents the imported curves with a given offset Y-axis value so that the curves are stacked and distinct from one another.

Mirror This should be ideally used to view two imported curves. One curve is inverted in the Y-axis and thus appears to mirror the other curve.

6. When you have made your selection, click on **OK**.

   If you selected the **Stack** option (see step 5), the **Shift Curves by Offset** dialogue is displayed. You can set the **Offset** value to increase or decrease the offset distance between the curves. To exclude a curve from the comparison, check on the associated check box to de-select it. Click on **OK**.

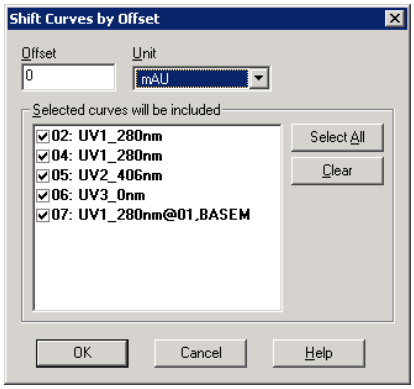


Fig 9-36. Stack Offset dialogue.

7. Imported curves are displayed in either the source chromatogram or in a new chromatogram that you created.

8. Select the curves that you want to view in the **Chromatogram Layout** dialogue. Curves can also be scaled individually or all with the same scale using the **All with this unit** function in the **Chromatogram Layout** dialogue (see "Y-Axis" on page 192).

   If you stacked the curves and you want to change the stack offset, select **Shift offset...** from the shortcut menu and select a new **Offset** value. The individual curves can also be moved (see "Alternative B: Moving a curve using the Shift function" on page 228).

9. If desired, select the **Store in new chromatogram** box, and give the chromatogram a new name.

Alternative B: Importing curves using Open

Using the **File:Open:Curves** function, individual curves may be imported into the active chromatogram.

1. Ensure that the destination chromatogram for the imported curve(s) is active on the screen.

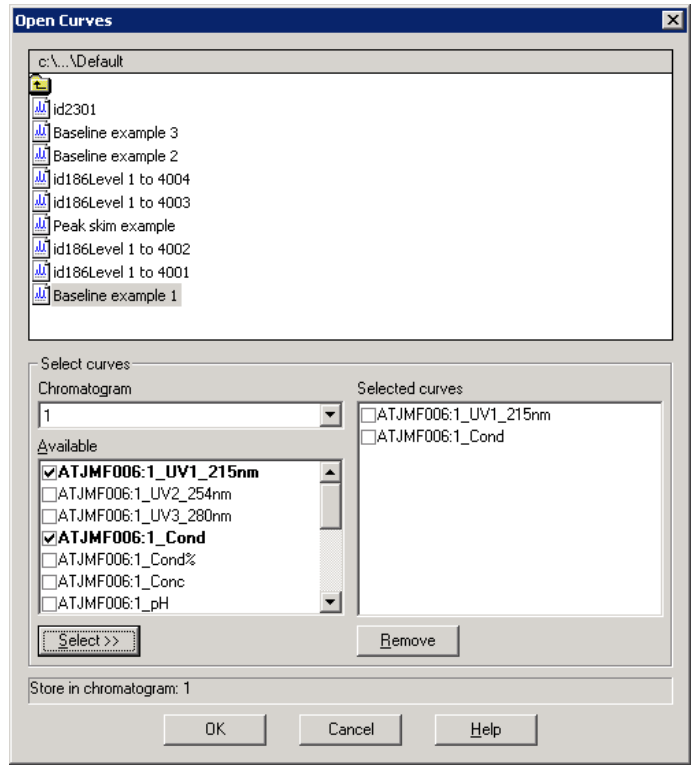


Fig 9-37. Open Curves dialogue.

2. Select **File:Open:Curves...**. The **Open Curves** dialogue is displayed.

3. Select the folder in which to search for curves.

4. Click on the result file of choice and, where appropriate, the specific chromatogram containing the desired curve. The chosen chromatogram and curves contained therein will be listed.

5. Select the desired curve and click on the **Select** button. The selected curve will now be displayed in the **Selected curves** list. To remove a curve from the **Selected curves** list, check the check box, then click on **Remove**.

6. If you want to choose more curves from other chromatograms repeat steps 3-4. When all the desired curves have been selected, click on **OK**.

7. Restore the chromatogram window and open the **Chromatogram Layout** dialogue. Select the curves that you want to view. Curves can be scaled individually or all with the same scale using the **All with this unit** function (see "Changing and fixing the axes" on page 192).

Alternative C: Copying curves into one chromatogram

Curves can be copied between chromatograms present in the Evaluation desktop. For effective comparison of curves, it is suitable to transfer all relevant curves to a single chromatogram. This is best achieved by:

- creating a new chromatogram using **File:New:Chromatogram...**, and copying curves into it from other chromatograms, or,
- copying an existing chromatogram using **Edit:Copy:Chromatogram...** and importing more curves into it, or,
- copying curves into the **Temporary** chromatogram (see "Temporary chromatogram" on page 186). You can perform evaluations in the **Temporary** chromatogram and transfer the final curves to other destination chromatograms. The unwanted contents remaining in the **Temporary** chromatogram can then be removed using **Edit:Clear Temporary Chromatogram**.

To copy curves:

1. Select **Edit:Copy:Curves...**.

2. Select the source chromatogram and the curve of interest. Select the target chromatogram. Click on the **Copy** button to effect the copy. Stay within the same dialogue to repeat this step for as many other curves you want, from the same or different chromatograms. When you have copied all desired curves, click on **Close**.

3. Open the destination chromatogram and access the **Chromatogram Layout** dialogue. Select in the **Chromatogram Layout** dialogue the curves that you want to view. Curves can be scaled individually or all with the same scale using the **All with this unit** function (see "Changing and fixing the axes" on page 192).

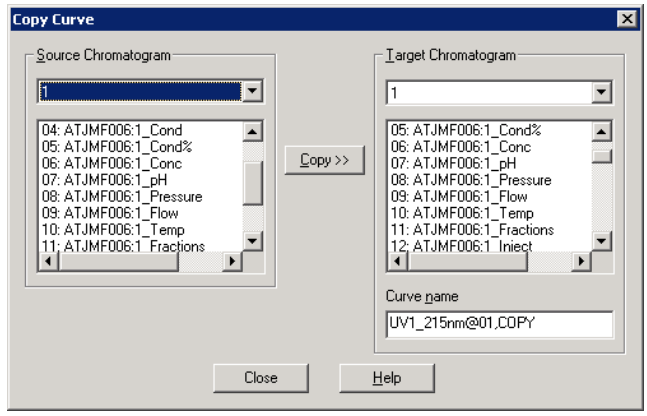


Fig 9-38. Copy curve dialogue.

9.4.5 Stacking and stretching curves

Several tools are available to stack and stretch curves from different runs to better visualise the differences. These tools are normalising curves, shifting curves and stretching curves. These allow you to manually reproduce the **Stack** and **Mirror** functions associated with the **Open to compare:Curves** operation (see "Alternative A: Import chromatograms using Open to compare" on page 216), and more besides. If the curves have been stacked with the **Open to compare curves** operation and you want to change the stack offset, the easiest way is to repeat the operation with another offset. The curves can also be stacked and stretched individually as described below. The operations presented below all require the curves to be present in one chromatogram (see "Comparing curves" on page 220).

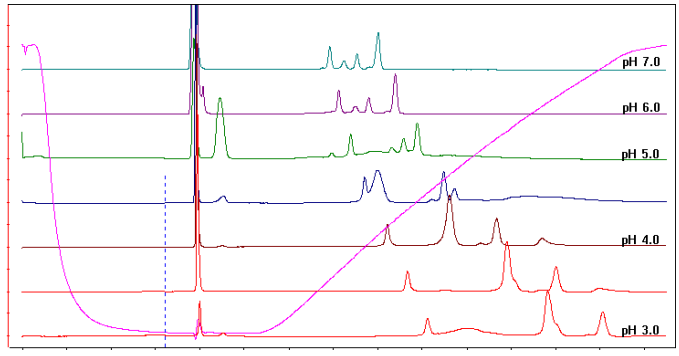


Fig 9-39. Stacking curves.

Alternative A: Stacking and stretching curves using the normalise function

The simplest method to align curves with respect to the X-axis or the Y-axis for easier visualisation, is to use the normalise function.

To select the curve to be moved within a chromatogram:

1. Select **Operations:Normalise**. The **Normalise** dialogue is displayed.

2. Select the curve to be normalised and a reference curve to be normalised against. For example, if you want to stack curves, select the curve at the bottom of the stack to be normalised against and the curve to be moved as normalised. Click on **OK**.

3. The **Normalise** window is displayed. A box surrounds the curve selected to be normalised.

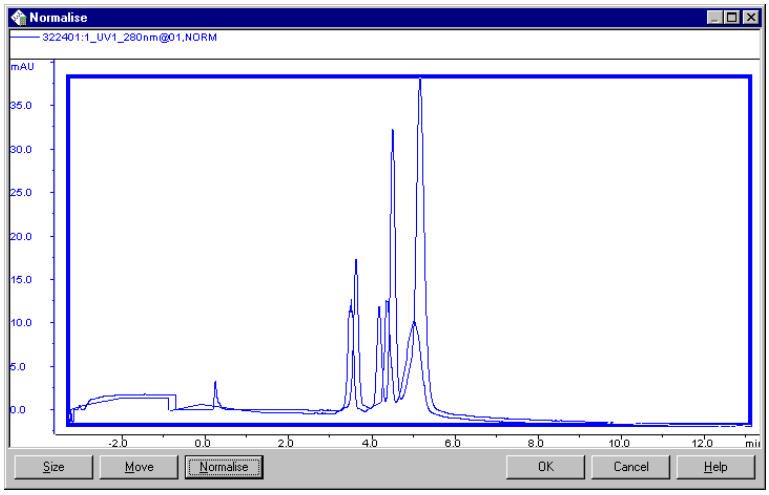


Fig 9-40. Normalise window.

You can now use the following functions:

Size Allows the selected curve to be stretched along its Y-axis or X-axis. Click on **Size** and then drag the coloured box either along its Y-axis or X-axis. This is useful for comparison of curves with, for example, different gradient lengths.

Move Allows the selected curve to be moved to any position on the chromatogram. Axes are automatically re-scaled to accommodate the new positioning. This function is useful for stacking curves. Click on **Move** and then move the curve with the mouse pointer. Click on the mouse button when the curve is in the correct position.

Normalise The curve to be normalised will be adjusted to the help curve. Thus, the height of the highest peak on both curves will be the same and will occur at the same retention point. The curve to be normalised is automatically moved along the X-axis and stretched along the Y-axis.

4. When all operations have been performed, click on **OK** to save the new normalised curve. Open the **Chromatogram Layout** dialogue to select the normalised curve for viewing.

5. Repeat the procedure for all curves you want to stack or stretch.

Alternative B: Moving a curve using the Shift function

If more precise positioning of curves is required, then the shift function should be used. This function is similar to **Normalise:Move** except that each curve is repositioned by a precise value instead of by eye and the instruction logged in the evaluation log.

1. Select **Operations:Shift**. The **Shift** dialogue is displayed. Select the curve to be shifted.

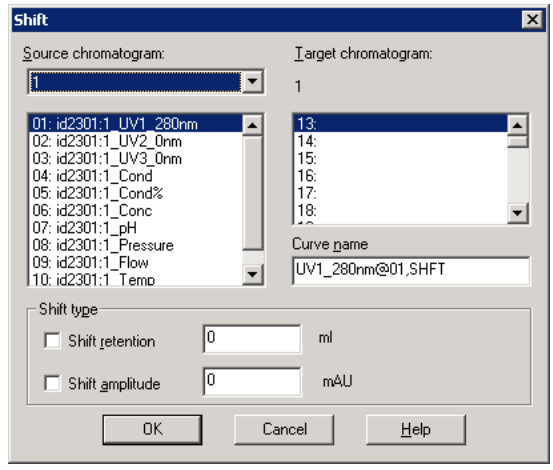


Fig 9-41. Shift dialogue.

2. Select the axis along which the shift is to be made, i.e. along the X-axis (**Shift retention**) or the Y-axis (**Shift amplitude**). Enter the shift value and click on **OK**.

Alternative C: Stretching and shrinking a curve using multiply

Curves can be stretched or shrunk in the x or y plane using the multiply function. This function is similar to **Normalise:Size** except that each curve is repositioned with precise numbers instead of by eye and the instruction logged in the evaluation log.

1. Click on **Operations:Multiply** and select the curve to be multiplied.

2. Select (check) the appropriate axis for multiplication, either **Multiply retention** and/or **Multiply amplitude**.

3. Insert the appropriate multiplication factor and click on **OK**.

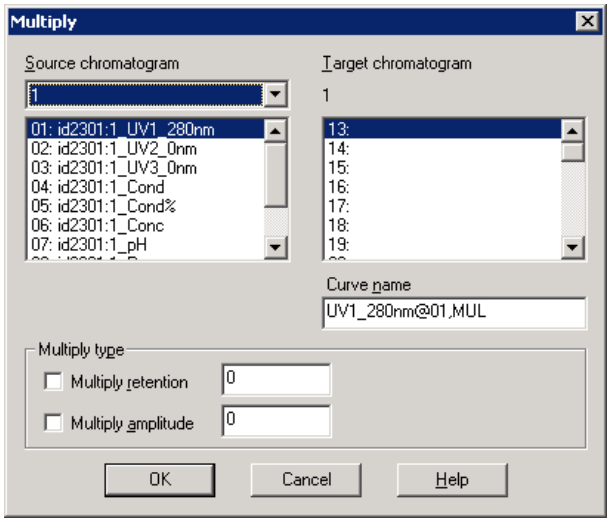


Fig 9-42. Multiply dialogue.

9.4.6 Mirror images of curves

A very useful way of comparing the features of two curves is to produce a mirror image of one curve. To achieve this:

1. Select **Operations:Multiply**.

2. Select the desired curve to be mirrored and select **Multiply amplitude** in the **Multiply type** field.

3. Type in a multiplication integer of -1 and click on OK.

4. Shift the mirror image curve downwards for an improved presentation (see Alternative B above).

Now the mirror image of the original curve will be displayed in the active window. Select/deselect for the other curves wanted in the active chromatogram window in the **Chromatogram Layout** dialogue.

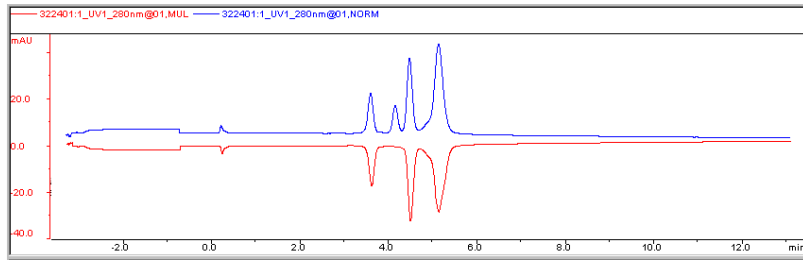


Fig 9-43. Two curves displayed in mirror image.

9.5 Electronically signing results

To electronically sign a result file, select **File:Sign Result**. The **Sign the Result** dialogue is opened.

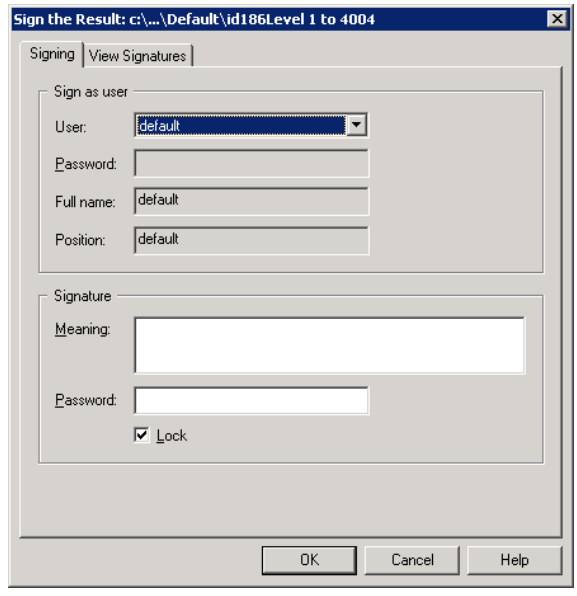


Fig 9-44. Sign the Result dialogue.

1. The **Sign as user** field shows the properties of the current user. In most instances, you will want to use the user shown in the **User** drop-down list box, but it is also possible to select another user from those available on the list.

2. In the **Meaning** field, provide a short text description explaining the meaning behind the signature (e.g., "Peak integration performed").

3. Type your user password in the **Password** field, and if desired, check the **Lock** box to permanently lock the result file from further changes.

Note: Locking the signature should only be done when you are sure that no further modifications to the file will be necessary.

4. The **View Signatures** tab provides a list of all signatures associated with the current method. Information on this tab is for viewing purposes only and may not be changed.

5. Within either the **Signing** or **View Signatures** tabs, click on **OK** to complete the signature process.

9.6 Saving results

Any changes to chromatograms, including all new created curves and all imported or created chromatograms, can be saved in either of two ways, using:

- **File:Save** or the **Save** toolbar icon, which saves all changes in the original result file, or
- **File:Save as**, which allows you to create a new result file in the specified target folder.

Note: All curves created during the manipulations will also be saved. This may not always be desirable. Before saving, remove unwanted curves from a chromatogram using **Edit:Delete:Curve**. The original curves can never be deleted.

9.7 Printing active chromatograms

To print out the open chromatogram(s) select **File:Print** or click on the **Print** toolbar icon. If you want to print out several chromatograms ensure that these are open on the workspace before selecting **File:Print**.

A dialogue appears allowing you to select the print format and (if applicable) the number of chromatograms in each column and row in

the printed document. It is also possible alter line thickness and present landscape views by selecting the checkboxes. Click on **Preview** to view the page layout before printing.

Click on **OK** to print out the chromatograms.

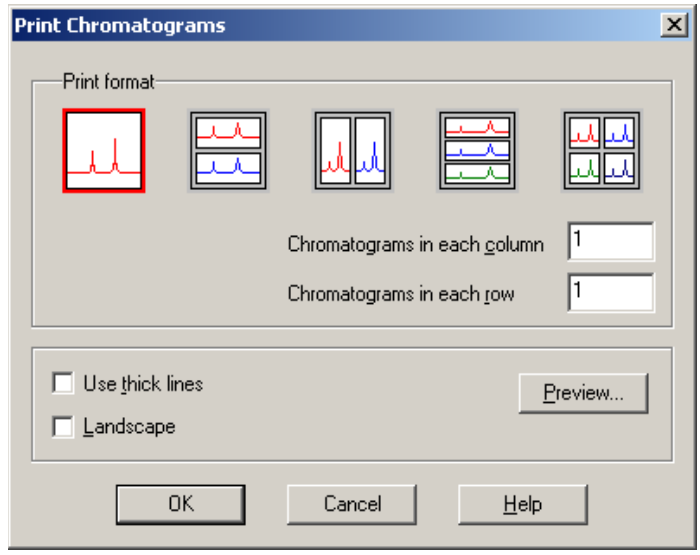


Fig 9-45. Print Chromatograms dialogue.

If the Documentation is open, click on the **Print** icon to print any components in the documentation.

The **Preview** button opens the **Customise Report** window, allowing for custom set-up of the print out.

Chromatograms can also be printed from the **File:Report** dialogue (see “Printing reports” on page 232).

9.8 Printing reports

The Evaluation module provides you with extensive tools with which to create detailed reports. You can create and save report formats based on either **Standard** layouts or on **Customised** layouts.

To open the report generator interface select **File:Report** or click on the **Report** toolbar button and the **Generate Report** dialogue is displayed.

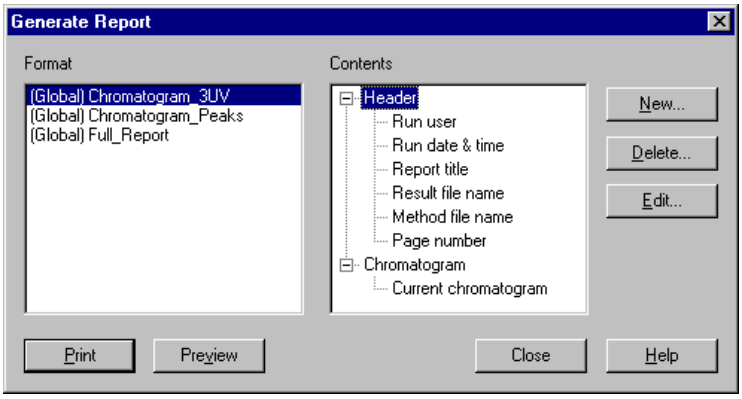


Fig 9-46. Generate Report dialogue.

In the dialogue you can see the available formats in the **Format** field. You can select a format from the list and directly apply it. Alternatively you can create a new report format or edit the existing formats.

Note: A report format saved with the **Current chromatogram** does not necessarily print the actual chromatogram as it appears on the screen in the Evaluation workspace.

If you want to print a number of results with the same report format, create a procedure to print one result and then perform a batch run for the required results (see "Batch runs" on page 298).

9.8.1 Creating a new customised report format

You can create your own report format and save it for later use. The customised report interface allows you to choose from a variety of objects including chromatograms, methods, documentation, free text and more. Moreover, you can decide the placing, alignment and sizing of the objects according to the various options or else apply free placement and sizing.

To access the customised report interface, click on the **New** button in the **Generate Report** dialogue to display the **Create New Report Format** dialogue. Click on the **Customised format** radio button (the default setting), and then on **OK**. The **Customise Report** window is displayed.

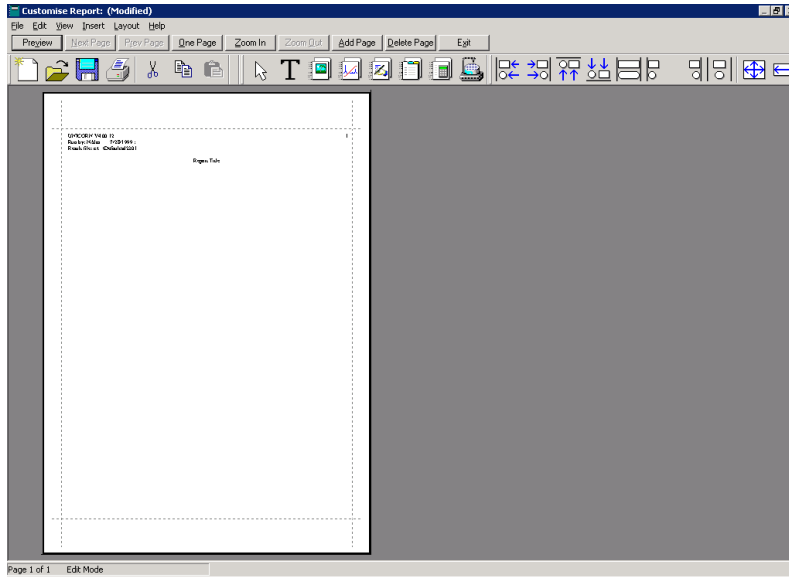


Fig 9-47. Customise Report window.

Adding and deleting pages

To add new pages to the report click on the **Add Page** button. A new page is added after the last page.

To delete a page while in single-page mode, click on the **Delete Page** button. Confirm the deletion. To delete a page in dual-page mode, click on an object on the page and then click on the **Delete Page** button. Confirm the deletion.

Adding objects

To add an object to the report:

1. Select an object from the **Insert** menu or click on the appropriate toolbar icon.

2. Move the mouse pointer into the page area of the window. You will notice that the pointer has an additional symbol according to the object type you selected to insert.

3. Press down and hold the left mouse button and drag out a box to the desired size. Release the mouse button. A dialogue is displayed specific to the type of object inserted. Make the appropriate

selections in the dialogue and then click on **OK** to view the inserted object.

The various object types and dialogues are as follows:

Free text

The **Setup Free Text** dialogue is used to define the desired text and the settings for the free text object.

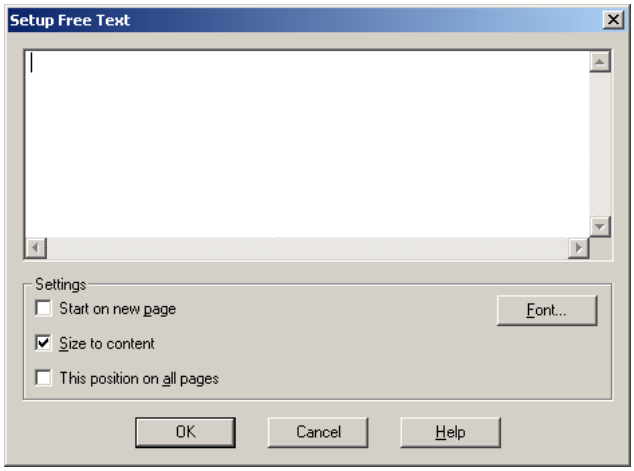


Fig 9-48. Setup Free Text dialogue

In the dialogue you can:

1. Enter text in the edit field.
2. If appropriate insert the text box on a new page by checking **Start on new page**.
3. Automatically size the free text box by checking **Size to content**.
4. If appropriate check the **This position on all pages** box to insert the text box in the same position on all pages. This is useful for setting texts in headers and footers.
5. Select the font type, style, colour and size by clicking on the **Font** button.
6. Click on **OK** once you have made your selections. The free text object is inserted.

Picture

The **Picture** dialogue is used to define the settings for picture object. This is useful when placing logos, pictures, or other figures in the report.

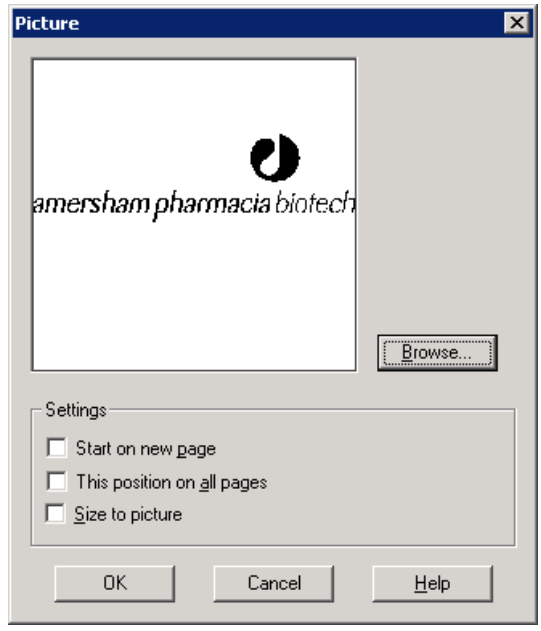


Fig 9-49. Picture dialogue.

1. Use the **Browse** button to locate the desired picture file (.bmp, .emf, .jpg, .tif). The currently selected picture is then previewed in the dialogue.

2. Check on the desired **Settings** options to format the picture.

3. Click on **OK** to set the picture in the report.

Chromatogram

The **Setup Chromatogram** dialogue is used to define the settings for the chromatogram object.

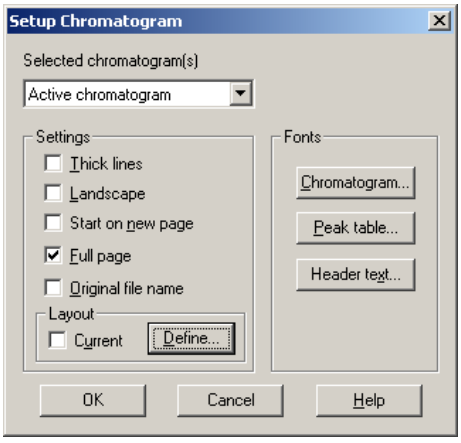


Fig 9-50. Setup Chromatogram dialogue

1. Select which chromatogram(s) to insert from the **Selected chromatogram(s)** drop-down list:

Active chromatogram	This inserts the chromatogram that is currently active in the Evaluation window.
All chromatograms	This inserts all chromatograms contained within the Evaluation window.
1, 2...etc.	This inserts the specific chromatogram corresponding to the selected number/name.

2. Check the appropriate **Settings** for the inserted chromatogram(s). You can select to view and print the chromatograms with **Thick lines**, view the chromatogram(s) in **Landscape** orientation, insert the chromatogram(s) so that they **Start on new page**, and/or show a chromatogram on a **Full page**. The **Original file name** can also be inserted on the graph.

3. To define the layout for the chromatogram(s) click on the **Define layout** button. The **Report Chromatogram Layout** dialogue is displayed. Make the appropriate selections in the various tabs for the report. If your chromatogram contains curves with different Y-axis units, click on the **Y-Axis** tab and select the appropriate curves within each of the axis drop-down list boxes.

Note1: Selections made in this layout only affect the report and not the view of the chromatograms in the Evaluation window.

Note2: Scouting variables and other appropriate variables can be selected for the chromatogram header, for example, if you wish to mark the chromatogram with the sample ID when using the autosampler.

Click on **OK** when you have made your selections to return to the **Setup Chromatogram** dialogue.

4. If appropriate change the characteristics of the **Fonts** by clicking on the buttons **Chromatogram**, **Peak table** and/or **Header text**.

5. When you have made your selections for the chromatogram(s) click on **OK** in the **Setup Chromatogram** dialogue. The chromatogram(s) are now inserted into the report.

Method

The **Setup Method** dialogue is used to define the settings for the method object.

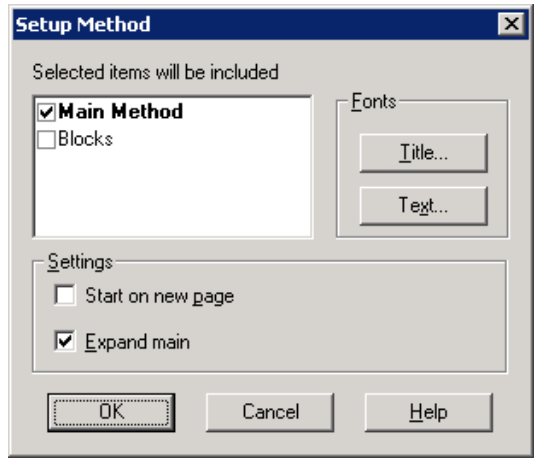


Fig 9-51. Setup Method dialogue.

1. Select the items to be included:

   Main Method This is the method on which the run was based.

   Blocks These are the blocks that were used in the method.

2. Select as appropriate for the inserted method to **Start on new page** and/or to **Expand main** to show the expanded view of the method.

3. If appropriate change the characteristics of the **Fonts** by clicking on the buttons **Title** and/or **Text**.

4. When you have made your selections click on **OK** in the **Setup Method** dialogue. The method is now inserted into the report.

Documentation

The **Setup Documentation** dialogue is used to define the settings for the documentation object.

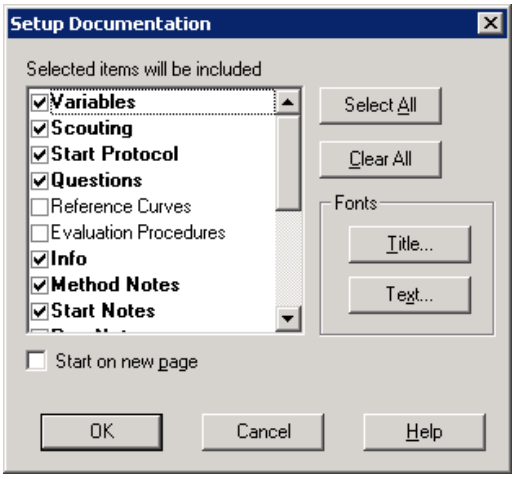


Fig 9-52. Setup Documentation dialogue.

1. Check the items to be included from the list box. To clear the current selection click on **Clear**. To select everything click on **Select all**.

2. If required select **Start on new page**.

3. If appropriate change the characteristics of the **Fonts** by clicking on the buttons **Title** and/or **Text**.

4. When you have made your selections click on **OK** in the **Setup Documentation** dialogue. Details from the selected documentation menu are now inserted into the report in the documentation object.

Evaluation log

The **Setup Evaluation Log** dialogue is used to chose the settings for the evaluation log object.

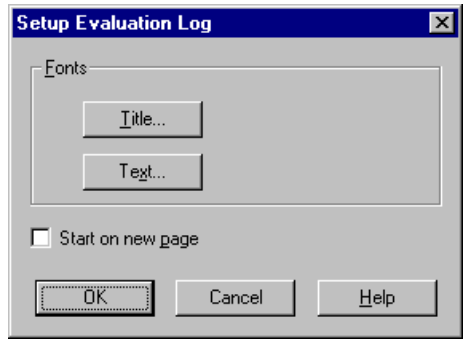


Fig 9-53. Setup Evaluation Log dialogue.

1. If appropriate change the characteristics of the **Fonts** by clicking on the buttons **Title** and/or **Text**.

2. If required select **Start on new page**.

3. When you have made your selections click on **OK**. The evaluation log is now inserted into the report.

Quantitate and Molecular Size

This option is only available if the Analysis module has been purchased and installed.

The **Setup Quantitate** dialogue is used to define the settings for the Quantitate and Molecular Size object.

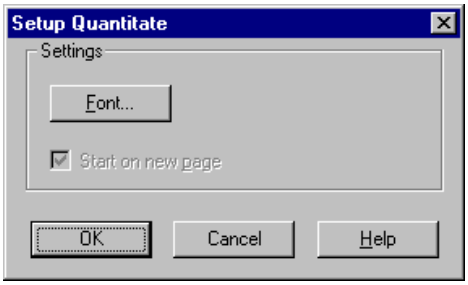


Fig 9-54. Setup Quantitate dialogue.

1. If appropriate change the characteristics of the **Fonts** by clicking on the buttons **Title** and/or **Text**.

2. When you have made your selections click on **OK**. The Quantitate and Molecular Size object is now inserted into the report.

Frac-950

The **Setup Frac-950** dialogue is used to define the settings for the Frac-950 object.

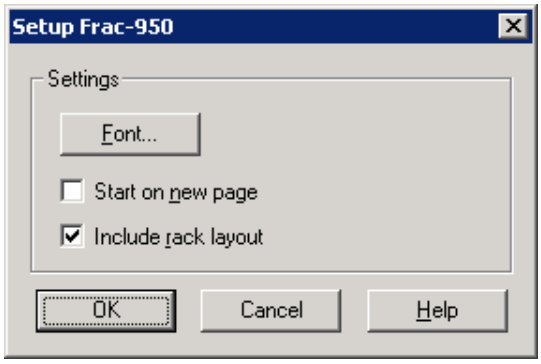


Fig 9-55. Setup Frac-950 dialogue.

1. Click on the **Font** button to set the font for the text.

2. The **Include rack layout** check box is checked by default. This will display the rack layout used in the run.

3. Click on **OK** to set the Frac-950 object into the report.

Moving and resizing objects

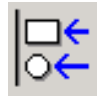
Objects can be freely moved, or moved and sized according to the various commands in the **Layout** menu or toolbar.

Note: Resizing the width of the objects with **Make same size** or **Make same width** can only be performed on chromatograms, free text and pictures.

1. To select an object for moving or sizing, click on the **Select** toolbar icon and click on the object of interest. To select several objects hold down the <Ctrl> key while clicking on the objects.

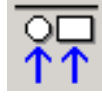
2. For free object placement of an object hold down the left mouse button and drag the object to its new position. Similarly, to free size the object, click on one of the object border anchors either at the corners or in the middle of a border and drag the box to re-size it.

3. For defined placement and/or sizing of object(s), select from the following options:

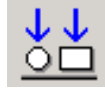


Matches the left alignment of all chosen objects to that of the highlighted object.

Matches the right alignment of all chosen objects to that of the highlighted object.



Matches the top alignment of all chosen objects to that of the highlighted object.



Matches the bottom alignment of all chosen objects to that of the highlighted object.

Adjusts the chosen object(s) to the left and right margins.

Adjusts the chosen object(s) to the left margin.

Adjusts the chosen object(s) to the right margin.

Adjusts the chosen object(s) to the centre of the page.

Adjusts the chosen objects to the same size as the highlighted object.

Adjusts the chosen objects to the same width as the highlighted object.

Adjusts the chosen objects to the same height as the highlighted object.

Viewing options

You have several viewing options available in the **View** menu or on the toolbar.

Preview/Edit	This toggles between looking at the print preview mode and edit mode
One Page/Two Pages	This toggles between viewing single pages or pairs of pages where there is more than one page
Next Page	This displays the next page or pair of pages where there is more than one page

Previous Page	This displays the previous page or pair of pages where there is more than one page
Zoom In	This increases the magnification of the view.
Zoom Out	This decreases the magnification of the view.

Changing the page layout

To change the page layout double click on the header region of the page to display the **Page Setup** dialogue.

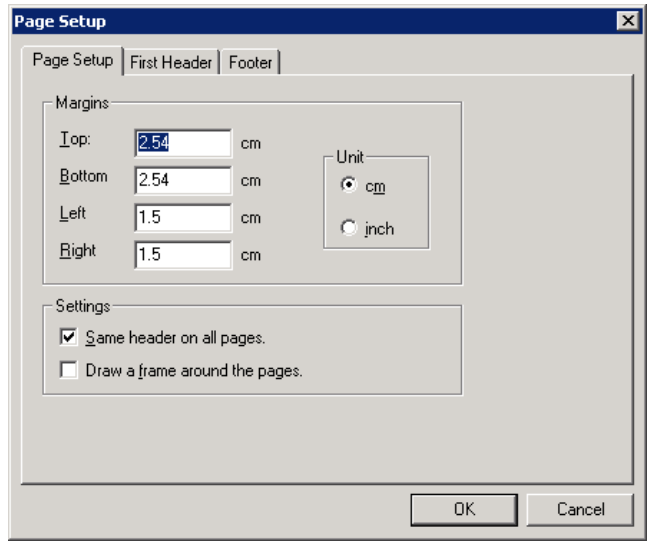


Fig 9-56. Page Setup dialogue, Page Setup tab.

There are three tabs for changing different aspects of the page layout:

Page Setup, which allows you to set the page **Margins** and the **Units**, **cm** or **inch**. You can check the option to have the **Same header on all pages** and also you can check the option to **Draw a frame around the pages**.

First Header, which allows you to select the components to be included in the header for the first page.

Note: If you have not selected the setting **Same header on all pages** on the **Page Setup** tab, then a fourth tab option, **Header**, is visible in the **Page Setup** dialogue. The **Header** tab allows you to select items to include in the headers of the individual pages, except for the first page.

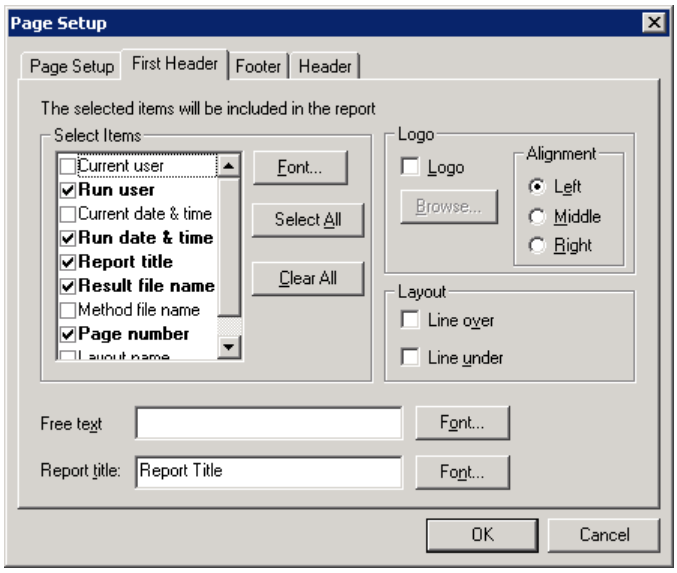


Fig 9-57. Page Setup dialogue, First Header tab.

1. Check the items to be included in the header. Click on **Select All** or **Clear** to facilitate your selection as appropriate.

2. If appropriate click on the **Font** button to alter the font characteristics.

3. Add **Free Text** and a **Report Title** as appropriate. You can also change the font style for these options.

4. If you have a logo in bitmap format this can be added to the header. Check the **Logo** option and then use the **Browse** function to locate your .bmp logo file.

5. Select the alignment of the logo, either **Left**, **Middle** or **Right**.

6. Select if you want a **Line under** or **Line over** the header.

7. Click on **OK** to implement the selection.

Footer, which allows you to select the components to be included in the footer.

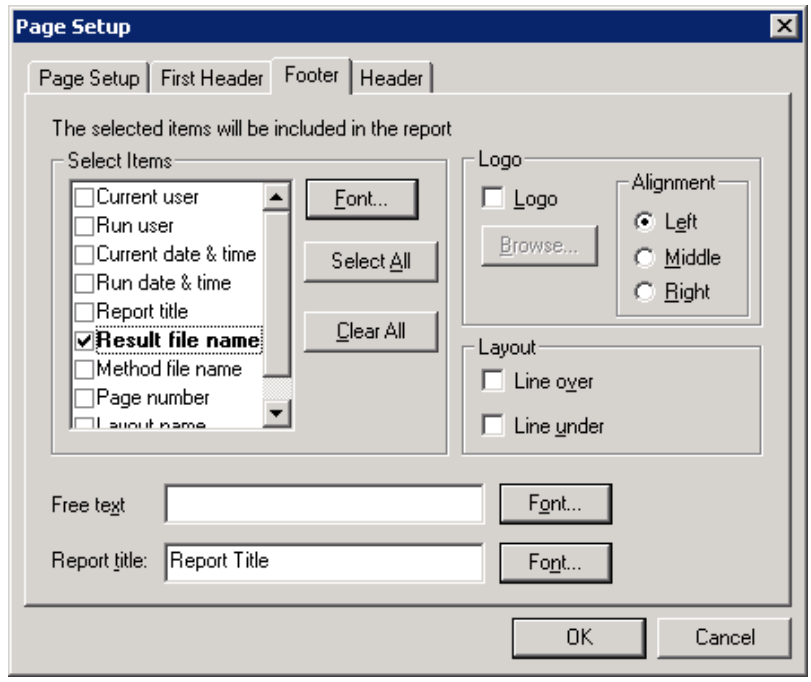


Fig 9-58. Page Setup dialogue, Footer tab.

Footer options are similar to those for **First Header** so you can have all information in either the header or footer or split the information between them as required.

Printing the report

To print the report select **File:Print** or click on the **Print** icon. Select the page range in the displayed dialogue and then on **OK**

Saving the report format

To save the report format:

1. Select **File:Save** or click on the **Save** toolbar icon. If saving for the first time the **Save Report Format** dialogue is displayed.

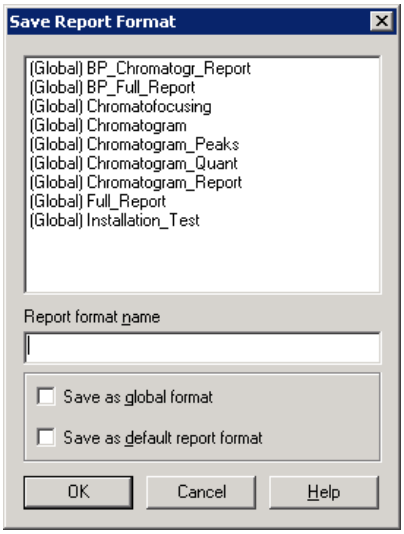


Fig 9-59. Save Report Format dialogue.

2. Enter a name for the format.

3. If you want the report format to be saved globally check the **Save as global format** option (if you have **Edit global lists** authorisation).

4. Click on **OK**.

Note: If you selected the **Save as default report format** option, the format name is changed to DEFAULT.

To save a copy of the format under another name, select **File:Save As** and enter a new name in the **Save Report Format** dialogue.

Exiting the Customise Report window

To exit the **Customise Report** window select **File:Exit** or click on the **Exit** button. You will be prompted to save unsaved formats.

When you have exited the window the newly created format is displayed in the **Generate Format** dialogue.

9.8.2 Creating a new standard report format

It you do not want to create a new report layout using a customised format you can instead use the fixed layout in the **Standard** report

formats. With **Standard** formats you can still select the objects that are included in the report and save the format for later use.

Selecting standard report options

1. Select the **New** button on the **Generate Report** dialogue. This prompts the **Create New Report Format** dialogue.

2. Choose **Standard format** and **OK**, and then use the tabs to select the report components within the displayed **Create Standard Report Format** dialogue. Note that the tabs for the standard report format are similar to the individual dialogues listed for objects in **Customised formats** (see "Creating a new customised report format" on page 233).

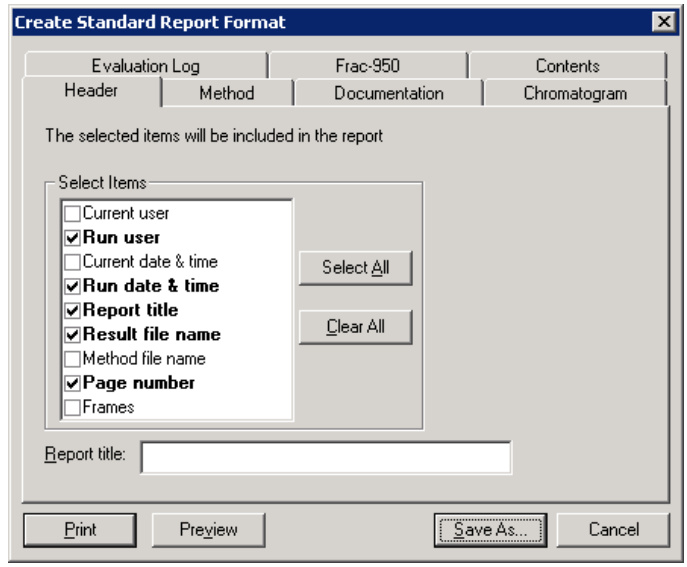


Fig 9-60. The Create Standard Report Format dialogue, Header tab.

3. You can preview the report contents by clicking on the **Contents** tab. Clicking on the + symbols next to the content headings reveals the contents of each section of the report.

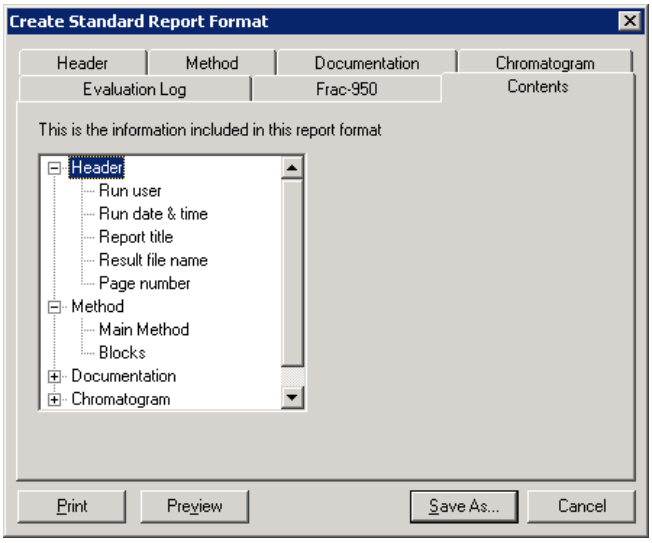


Fig 9-61. Create Standard Report Format dialogue, Contents tab.

Previewing and printing the report

Within any of the tabs on the **Create Standard Report Format** dialogue, you can preview the entire report printout by clicking **Preview**. Similarly, once you have made all of the desired adjustments to the report format, you can click on the **Print** button from within any of the tabs.

Saving the report format

1. Click on the **Save As** button.
2. In the displayed dialogue enter a name for the format.
3. If you want the report format to be saved globally check the **Save as global format** option (if you have **Edit global lists** authorisation).
4. If you want the format to be used as the default format check the **Save as default report format** option.
5. Click on **OK**.

Note: If you selected the **Save as default report format** option, the format name is changed to DEFAULT.

9.8.3 Modifying an existing report format

Another way of creating a new report format is to edit an existing format.

1. Select **File:Report** or click on the **Report** toolbar button and the **Generate Report** dialogue is displayed.

2. Select the report format of interest and click on the **Edit** button. Select either **Standard format** or **Customised format** and click **OK**.

Customised format

Choosing the **Customised format** button opens the **Customised Report** window. As these options are dealt with for creating a new customised report (see "Creating a new customised report format" on page 233), they will be treated here only briefly. To add formatting to the page layout:

1. Add new objects as desired. Alternatively you can **Cut**, **Copy**, **Paste**, or **Delete** objects using the shortcut menu.

2. Edit existing and new objects by moving them to new positions, resizing them or changing their properties. Change properties by clicking on an object with the shortcut menu. This generates a shortcut menu that allows you to the highlighted section.

3. You can also modify the contents of the highlighted report section by choosing the **Properties** command. From the shortcut menu, click on **Properties**, make the desired choices on the resulting dialogue and press **OK**.

4. Once the desired formatting choices have been made, clicking on the **Preview** button will display the page layout. Press the **Print** button to print out the report. Also you can save the modified format under a new name by selecting **File:Save As**.

Standard format

Choosing the **Standard format** button generates a second **Edit Standard Report Format** dialogue with tabs representing format options for the major sections of the report. The options within the various tabs are identical to those available when creating a new standard format (see "Creating a new standard report format" on page 247).

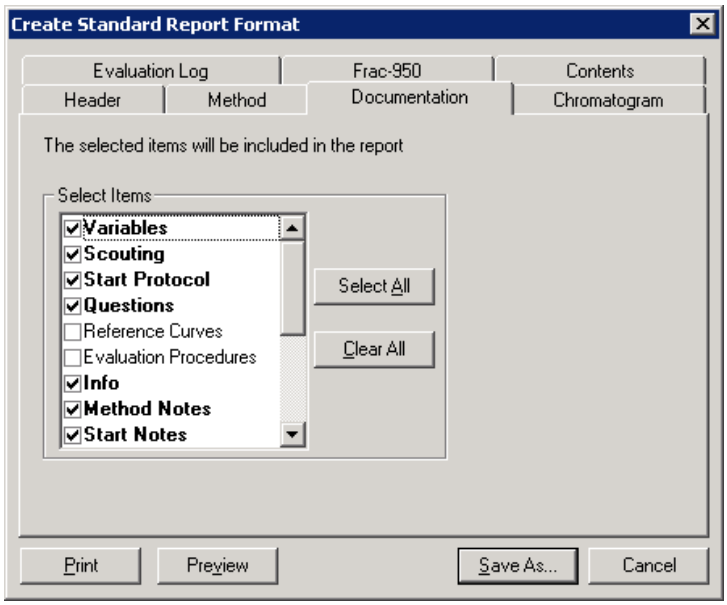


Fig 9-62. Create Standard Report Format dialogue, Documentation tab.

Once the desired formatting choices have been made, clicking on the **Preview** button will display the page layout. Press the **Print** button to print out the report. Also you can save the modified format under a new name by clicking on the **Save As** button.

9.9 Run documentation

The full documentation of a run is stored within the result file. Some of the documentation functions are described below.

- To open the **Documentation**, either select **View:Documentation** or click on the **View Documentation** toolbar icon.
- To print documentation contents, click on the **Print** button in the **Documentation** dialogue (see "Printing active chromatograms" on page 231).
- The contents can be saved as a new method by selecting **Method** page and clicking on **Save as**.

Variables

The **Variables** tab lists the parameters that were used during the run.

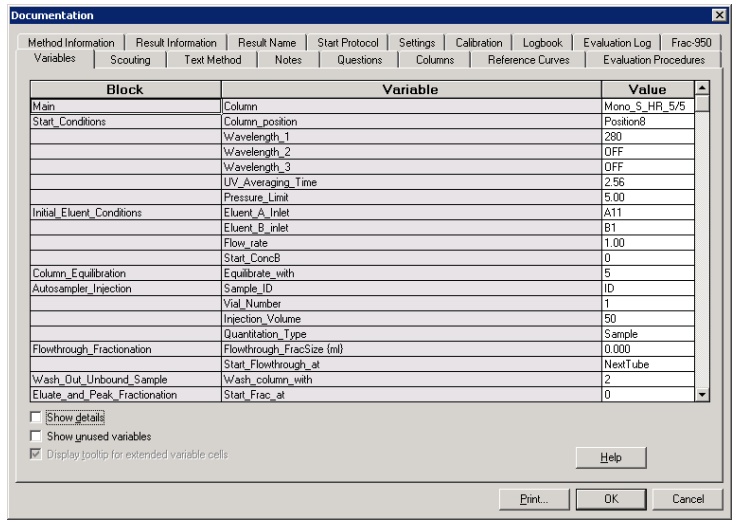


Fig 9-63. Documentation, Variables tab.

Scouting

This displays the whole scouting scheme, with the values for the current result file displayed in yellow cells.

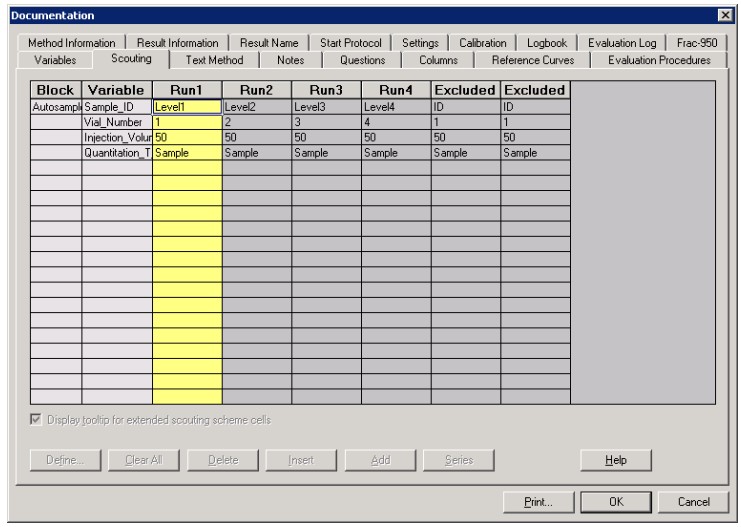


Fig 9-64. Documentation, Scouting tab.

Notes

This displays notes that you have made at various points during the run. You are also able to enter new comments in the **Evaluation Notes** tab.

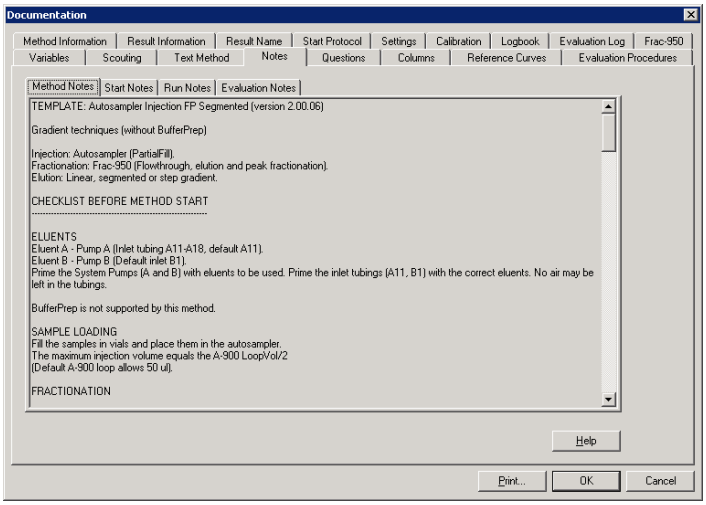


Fig 9-65. Documentation, Notes tab.

Calibration

This displays what system calibrations were made, when and by whom.

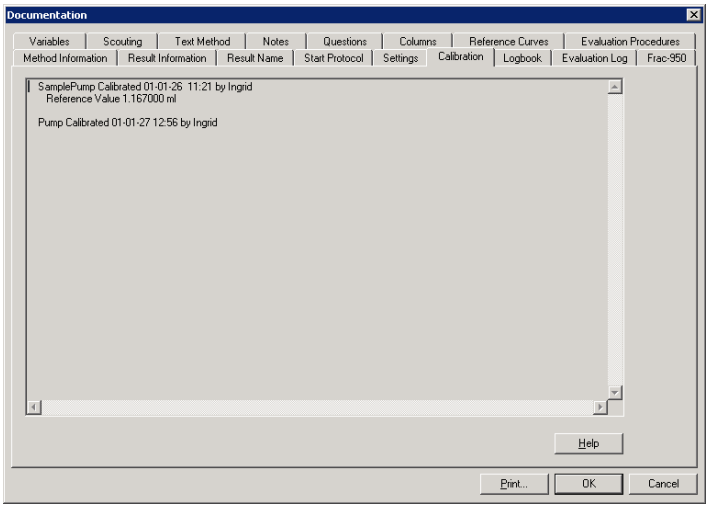


Fig 9-66. Documentation, Calibration tab.

Logbook

This displays what happened during a run. It is possible to view information concerning alarms, the method, manual changes during the run, errors and the system.

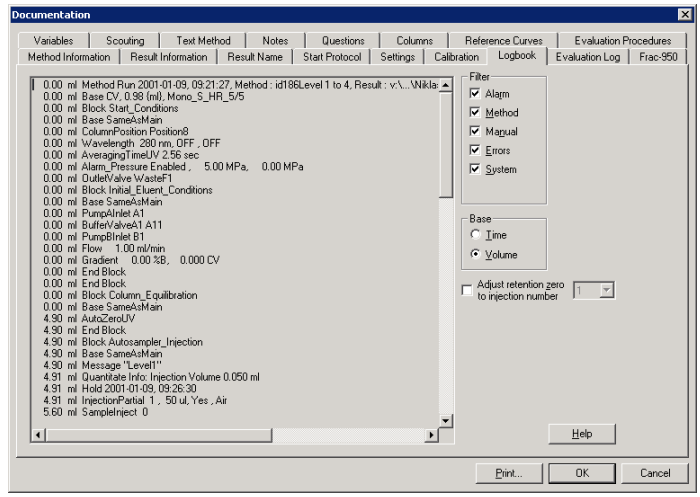


Fig 9-67. Documentation, Logbook tab.

Evaluation Log

This lists all of the evaluation operations that you have performed for the current result file for all sessions, including procedures executed at the end of the method.

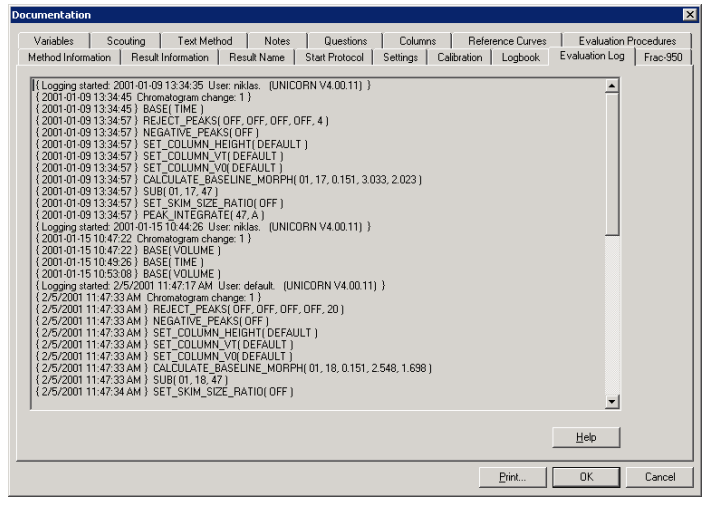

Documentation
Variables
Scouting
Text Method
Notes
Questions
Columns
Reference Curves
Evaluation Procedures
Method Information
Result Information
Result Name
Start Protocol
Settings
Calibration
Logbook
Evaluation Log
Frac-950
{ Logging started: 2001-01-09 13:34:35 User: niklas. (UNICORN V4.00.11) }
{ 2001-01-09 13:34:45 Chromatogram change: 1 }
{ 2001-01-09 13:34:45 } BASE(TIME)
{ 2001-01-09 13:34:57 } REJECT_PEAKS(OFF, OFF, OFF, OFF, 4)
{ 2001-01-09 13:34:57 } NEGATIVE_PEAKS(OFF)
{ 2001-01-09 13:34:57 } SET_COLUMN_HEIGHT(DEFAULT)
{ 2001-01-09 13:34:57 } SET_COLUMN_VT(DEFAULT)
{ 2001-01-09 13:34:57 } SET_COLUMN_V0(DEFAULT)
{ 2001-01-09 13:34:57 } CALCULATE_BASELINE_MORPH(01, 17, 0.151, 3.033, 2.023)
{ 2001-01-09 13:34:57 } SUB(01, 17, 47)
{ 2001-01-09 13:34:57 } SET_SKIM_SIZE_RATIO(OFF)
{ 2001-01-09 13:34:57 } PEAK_INTEGRATE(47, A)
{ Logging started: 2001-01-15 10:44:26 User: niklas. (UNICORN V4.00.11) }
{ 2001-01-15 10:47:22 Chromatogram change: 1 }
{ 2001-01-15 10:47:22 } BASE(VOLUME)
{ 2001-01-15 10:49:26 } BASE(TIME)
{ 2001-01-15 10:53:08 } BASE(VOLUME)
{ Logging started: 2/5/2001 11:47:17 AM User: default. (UNICORN V4.00.11) }
{ 2/5/2001 11:47:33 AM Chromatogram change: 1 }
{ 2/5/2001 11:47:33 AM } REJECT_PEAKS(OFF, OFF, OFF, OFF, 20)
{ 2/5/2001 11:47:33 AM } NEGATIVE_PEAKS(OFF)
{ 2/5/2001 11:47:33 AM } SET_COLUMN_HEIGHT(DEFAULT)
{ 2/5/2001 11:47:33 AM } SET_COLUMN_VT(DEFAULT)
{ 2/5/2001 11:47:33 AM } SET_COLUMN_V0(DEFAULT)
{ 2/5/2001 11:47:33 AM } CALCULATE_BASELINE_MORPH(01, 18, 0.151, 2.548, 1.698)
{ 2/5/2001 11:47:33 AM } SUB(01, 18, 47)
{ 2/5/2001 11:47:34 AM } SET_SKIM_SIZE_RATIO(OFF)
Help
Print...
OK
Cancel

Fig 9-68. Documentation, Evaluation Log tab.

Method information

The **Method Information** tab displays information about the method, such as method name, target system for creation and date of last change. Strategy information includes, strategy name, date and size. There is also a tab for **Signatures** associated with the method.

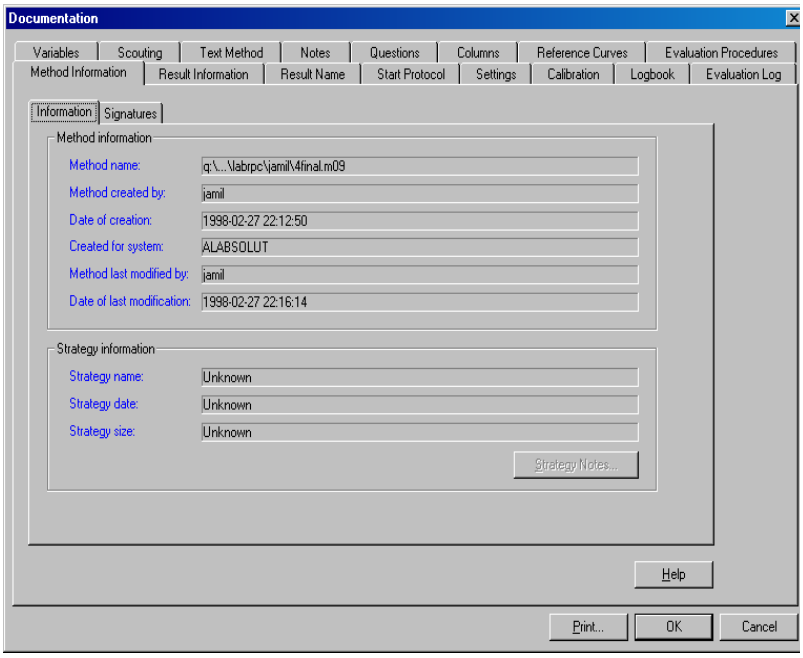


Fig 9-69. Documentation, Method information tab.

Frac-950

The **Frac-950** tab displays the setup parameters for the fraction collector. You can print out the selected settings by pressing the **Print** button, or return to the main Evaluation window by pressing **OK** or **Cancel**.

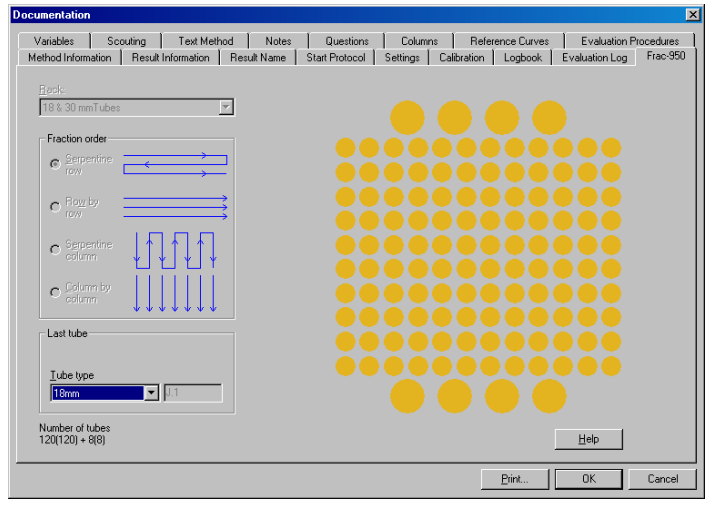


Fig 9-70. Documentation, Frac-950 tab.

Result information

The **Result information** tab displays information about the method, such as method name, target system for creation and date of last change. Strategy information includes, strategy name, date and size. The **Run Summary** tab summarises the run in terms of volume or time per block. There is also a tab for **Signatures** associated with the result.

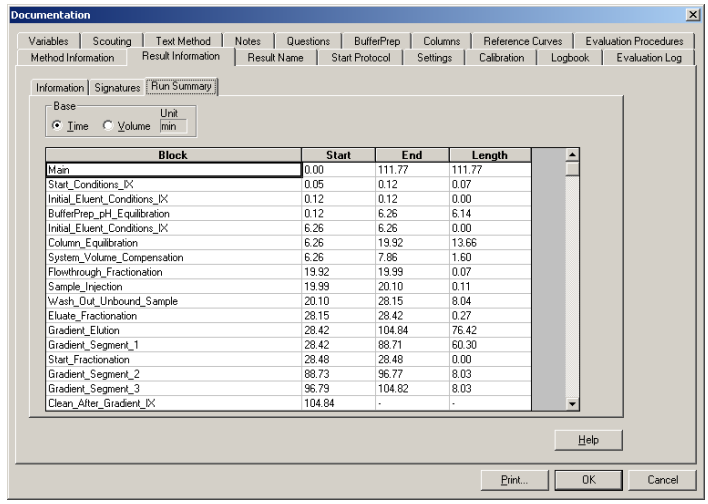


Fig 9-71. Documentation, Result Information tab.

9.10 Exiting Evaluation

If you want to quit from the Evaluation module of UNICORN, select **File:Exit**. You will then be asked if you want to save the results of the evaluation session that you have performed. If you answer **Yes**, the previous version of that result file will be unconditionally over-written.

10 Evaluating results

This chapter will mainly describe how to:

- integrate peaks
- automate evaluation operations
- export data and curves

10.1 Integrating peaks

Using peak integration, UNICORN allows you to identify and measure a number of curve characteristics including peak areas, retention times, and peak widths.

10.1.1 Baseline calculation for integration

Integrating peaks is divided into two steps: calculating the baseline and calculating peak areas. As a correct baseline is crucial for accurate calculation of peak areas, several ways of calculating the baseline are available in UNICORN:

- Using the **Calculate baseline** instruction for automatic calculation of the baseline, which gives, in most cases, a very accurate measurement. **Calculate baseline** is the most common method for calculating baselines. Baseline calculation can be performed using the Morphological algorithm or Classical algorithm (see sections below). For a description of the principles of baseline creation, see “Baseline calculation theory” on page 371.
- A blank run curve with the same chromatographic conditions as the corresponding sample can be used as the baseline for peak integration. Another approach that may improve the peak integration (if a blank run is available) is to first subtract the blank run from the source curve (see “Subtracting a blank run curve” on page 201) and then perform peak integration on the resulting curve using the **Calculate baseline** option.

  In addition to blank runs, it is possible to select any curve present in the current chromatogram, e.g. an edited baseline (see “Manually editing a baseline” on page 276), as baseline.

- Using a **Zero baseline**, i.e. no baseline subtraction at all.
- Reusing an already existing baseline for the selected curve by selecting the **Correlated baseline** option. This is the default alternative whenever possible.

10.1.2 Performing a basic integration

To perform a basic integration:

1. Select **Integrate:Peak integrate** or click on the **Peak Integrate** toolbar icon. The **Integrate** dialogue is displayed.

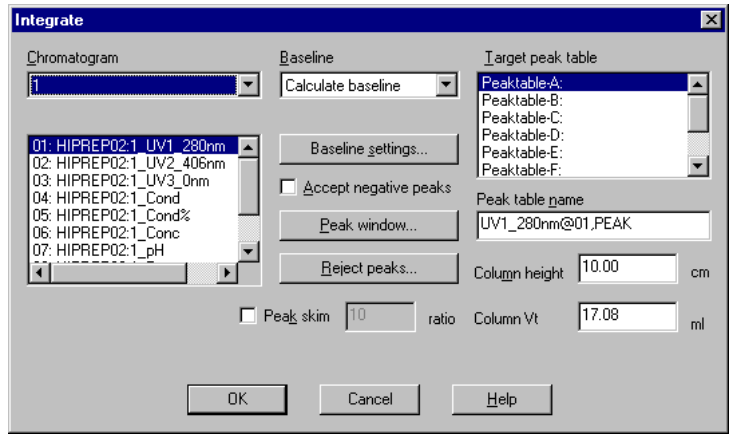


Fig 10-1. Integrate dialogue.

2. Choose the source curve to be analysed, usually the first UV curve, and a peak table destination from the peak table list. Any chromatogram can contain up to eight peak tables, designated A-H.

3. Make the appropriate **Baseline** selection from the above-mentioned possibilities. The **Calculate baseline** option with the default settings for the parameters is the most common choice.

Note: There are two choices of algorithm for baseline calculation, **Morphological** and **Classic**. The default setting is **Morphological** (for more details, see "Optimising peak integration" on page 262).

4. Click on **OK** to perform the peak integration when you are satisfied with your selections.

Following integration, the peaks in the chromatogram will be automatically labelled with their respective retention times. The start and end point of each peak will be marked by drop-lines.

The peak table will be displayed underneath the active chromatogram.

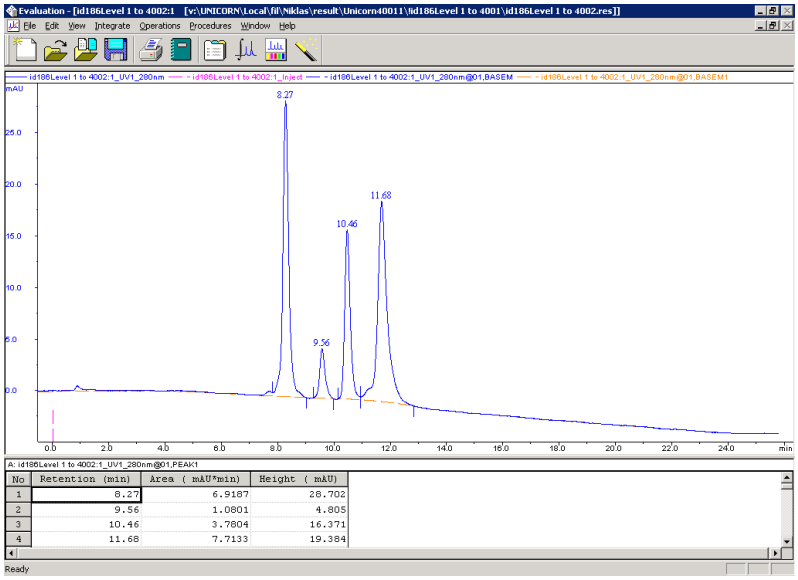


Fig 10-2. The results after peak integration.

In addition to peak areas, several other peak characteristics such as retention time and peak width are automatically calculated. The characteristics displayed in the peak table may be selected in the **Chromatogram Layout** dialogue, **Peak Tables** tab.

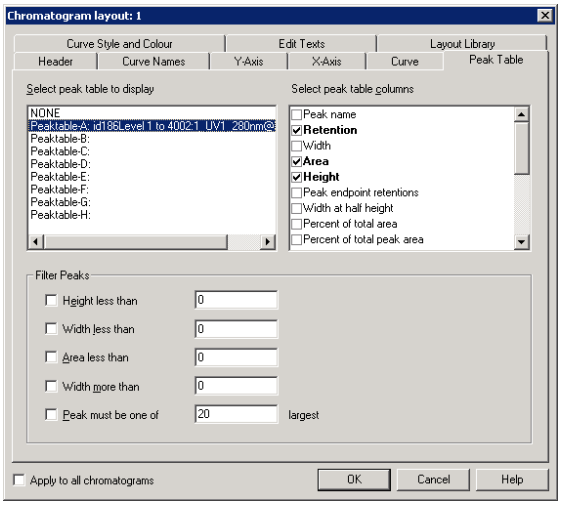


Fig 10-3. Chromatogram Layout dialogue, Peak Table tab.

Select the options that you want to be displayed from the **Select peak table columns** list. Most characteristics are automatically calculated for each integrated peak when the peak integrate function is used, although only the selected items will be displayed in the peak table.

Changing peak labels

As alternatives to using retention times as peak labels, the peaks can be sequentially numbered or be marked with specific identification tags. The choice of label type is made in the **Curve Style and Colour** tab in the **Chromatogram Layout** dialogue (see "Changing the colour and style of curves" on page 191). If you do not want to view the peak labels, e.g. for presentation purposes, select the **No peak label** option.

Filtering peaks from view

It is possible to temporarily remove peaks from display in a peak table based upon the criteria you determine.

1. In the **Chromatogram Layout** dialogue, click on the **Peak Table** tab.

2. Select (check) the filter criteria in the peak table and specify the values used to filter the peaks, i.e. the minimum height, width and area, the maximum width as well as a specified number of the largest size peaks. Click on **OK**.

If you later want to include the peaks again you have to deselect the options. The difference between **Filter peaks** and **Reject peaks** is that the latter function permanently excludes peaks from the integration and affects the calculation of total peak area etc.

10.1.3 Optimising peak integration

If the results from the peak integration are unsatisfactory, there are several possibilities to improve the results.

Morphological and Classic baseline calculation

You can use one of two baseline calculation algorithms depending on the type of peaks to be integrated.

- The **Morphological** algorithm is set as the default and gives the best results in curves with drifting baseline and peak clusters. Optimising baseline calculation using the **Morphological** algorithm is also relatively easy since there are only three baseline parameters, namely structure width, noise window and minimum distance between points (see "Optimising the baseline parameters using a morphological algorithm" on page 266).

- The **Classic** algorithm has long been used as the standard for calculating the baseline (see "Optimising the baseline parameters using a classic algorithm" on page 269). The **Classic** algorithm is particularly useful in integrating curves containing negative peaks. Thus, the **Classic** algorithm should be selected if the **Morphological** algorithm gives poor results from the presence of negative peaks or where quantitative data from negative peaks are important in the run.

To select the appropriate algorithm and change baseline settings:

1. Display the **Settings** or **Baseline Settings** dialogue, respectively, by (i) clicking on the **Baseline settings** button in the resulting **Integrate** dialogue, or (ii) selecting **Integrate:Calculate baseline**. These dialogues function identically. However, in the former case, the baseline is immediately used in a peak integration.

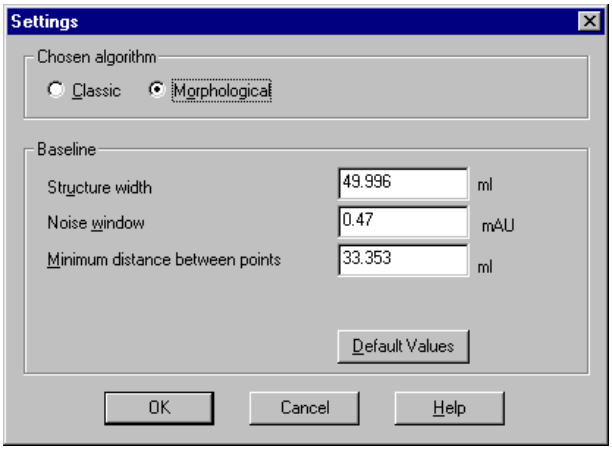


Fig 10-4. Settings dialogue.

2. Select the appropriate algorithm, **Classic** or **Morphological**.

3. Change the **Baseline** parameter values as appropriate.

Excluding peaks

It is possible to exclude peaks from integration based upon criteria you determine. Click on the **Reject peaks** command button in the **Integrate** dialogue. In the dialogue that is displayed, select (check) the criteria and parameters by which peaks will be excluded from the integration. You are able to define the minimum height, width and area, the maximum width as well as a specified number of the largest size peaks. The default criterion is to include only the 20 largest peaks.

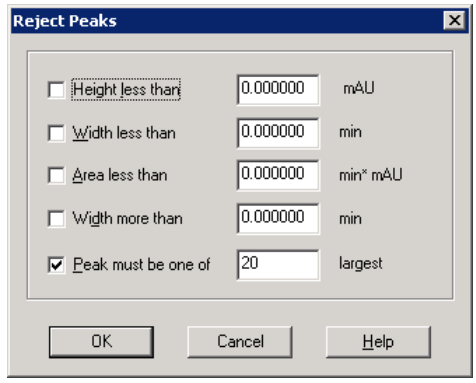


Fig 10-5. Reject Peaks dialogue.

Selecting part of a curve for integration

To select only a part of a curve for integration, click on the **Peak window** button in the **Integrate** dialogue. A chromatogram window will open containing the curve and two vertical cursor lines. These lines can be dragged to define a region between them that will be analysed. Alternatively, X-axis values for the **Left limit** and **Right limit** may be typed in. Click on **OK** to return to the main dialogue. The baseline will be calculated from the whole curve, but calculation of the areas beneath the peaks is only performed on the selected section of the curve. The default peak window includes the entire curve from the injection point.

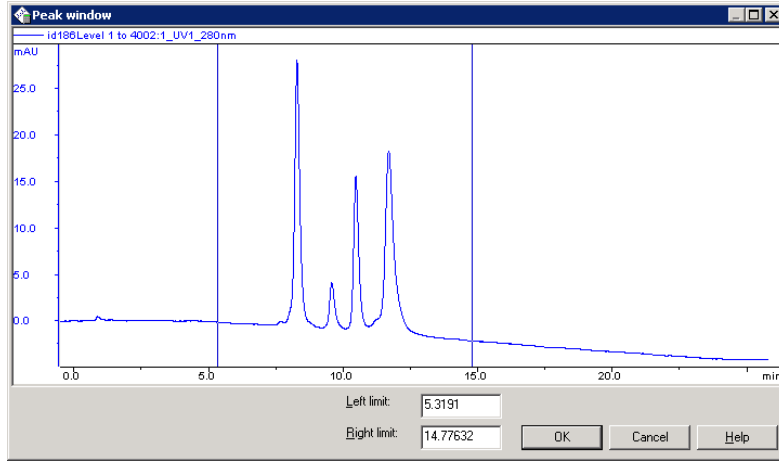


Fig 10-6. Peak Window chromatogram window.

Peak skimming

The area under a peak can be calculated either using drop-lines or peak skimming. Drop-lines are vertical marks that split two peaks at the valley. This is most commonly used for peaks of relatively similar size. In some circumstances, for example when a peak has a shoulder, use of a drop-line will cause too much area of the first peak to be lost to the peak that forms the shoulder. Thus, the skim peak function can be used when the smaller peak is skimmed off with a straight line starting at the valley between the peaks, and ending at the point on the other side of the smaller peak where the slope of the skim line is equal to the slope of the curve. In doing so, this skims the area under the second peak.

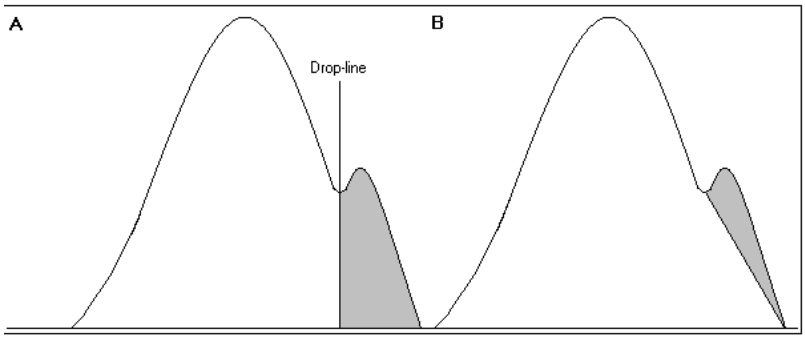


Fig 10-7. Illustration of how a drop-line (A) and a skimmed peak (B) affects the area under the main peak and the peak shoulder.

The **Skim peak** option can be checked in the **Integrate** dialogue. You can also set the **Ratio** value to determine when peak skimming should be applied to a peak instead of drop-lines (the default value for this ratio is 10). The ratio is based on the relationship:

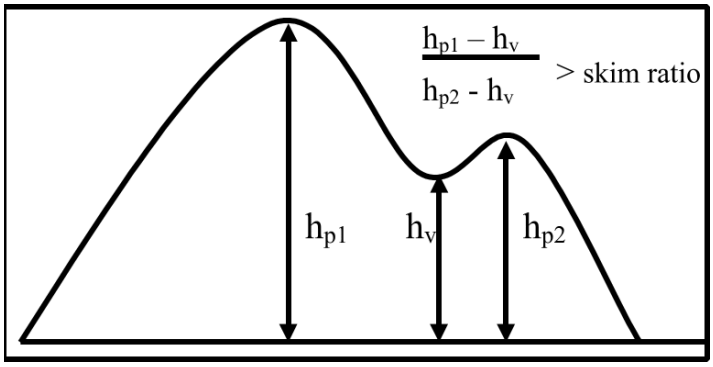


Fig 10-8. Skim peak ratio calculation.

Including negative peaks

If you want to include negative peaks in the integration, select (check) **Accept negative peaks** in the **Integrate** dialogue (or in the **Calculate baseline** dialogue). The negative peaks will be reported as negative areas in the peak table. By default, negative peaks are not included in the integration.

Manually editing the baseline

In the event that the automatic baseline calculation does not produce a satisfactory baseline, it is possible to edit the baseline manually by inserting and deleting baseline points. This is done with the **Integrate:Edit baseline** function (Manually editing a baseline).

Manually editing the peak table

The **Integrate:Edit peak table** function (Adjusting the peak limits) allows you to manually adjust the peak start and end points, split and join peaks.

10.1.4 Optimising the baseline parameters using a morphological algorithm

The first choice when trying to optimise the peak integration is to change the baseline parameters. For a brief description of the parameters and a suggested way of estimating settings for the **Morphological** baseline parameters from the source curve, see "Baseline calculation theory" on page 371.

The **Morphological** algorithm can be described in terms of a straight line that "strolls" along the chromatogram parallel to the X-axis. Data points for the baseline are created wherever the straight line touches the curve and the points are joined at the end to create a baseline.

Structure width

When optimising the baseline parameters using a **Morphological** algorithm, changes to **Structure width** will in most cases give the best improvement in results. **Structure width** determines the length of the straight line (see above), which is set to a default value of the widest peak on the chromatogram multiplied by 1.5. In situations with drifting baseline for a curve, the morphological baseline follows the curve faithfully. Subsequent subtraction of the baseline thus creates a curve with the baseline at a more even level.

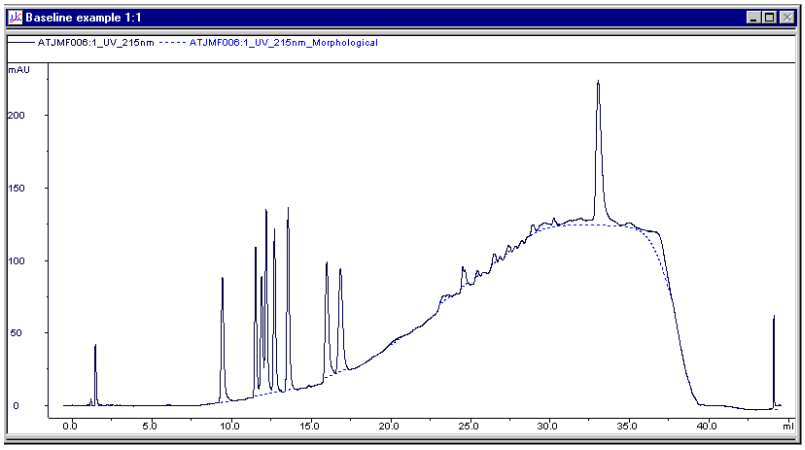


Fig 10-9. Fluctuating curve with a morphological baseline that follows the bases of the peaks at the different levels in the curve.

In some circumstances the default setting for the **Structure width** should be increased since a too low setting may result in a baseline that reaches too high up in the peaks of the curve (see figure below). This situation may arise when, for example, a wider peak is not recognised due to it containing a cluster of smaller peaks. The default **Structure width** is therefore set to a default value according to the largest width of the identified narrower peaks.

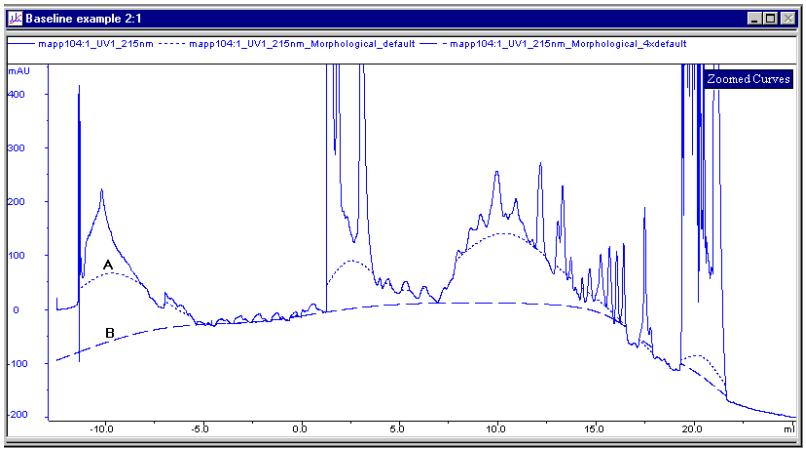


Fig 10-10. Chromatogram displaying a curve with two baselines; (A) default morphological algorithm settings and (B) morphological algorithm with increased structure width value.

Conversely, a too large **Structure width** value means that narrower peaks, especially in fluctuating curves, may not be properly followed. This may arise when an artifact in a curve is identified by the morphological algorithm as the widest peak and hence used to set the default **Structure width** value.

Minimum distance between points

The **Minimum distance between points** is a measure of the distance between the data points used to generate a baseline. The largest number of data points is produced at the slopes of the curves, and so by increasing the **Minimum distance between points** value fewer points will be collected on the slopes.

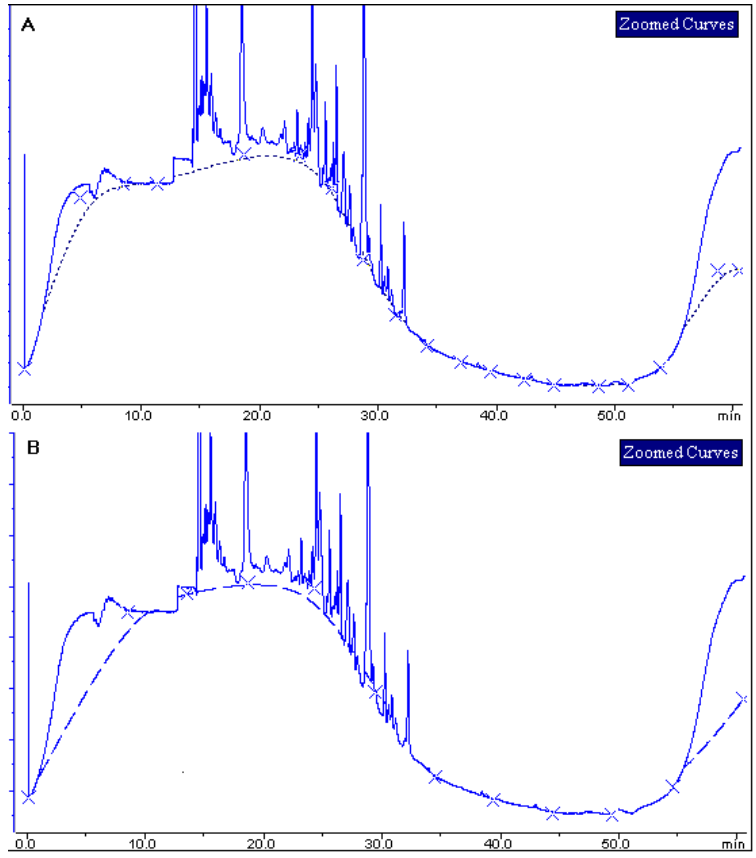


Fig 10-11. A curve in a chromatogram integrated using the morphological algorithm contains many data points when the Minimum distance between points parameter is set to a low value (A). The number of data points is reduced when the Minimum distance between points parameter is set to a higher value (B).

Noise window

Sometimes you get too many peaks after the peak integration, usually because noise on the baseline is erroneously detected as peaks.

The cause of this problem is that the **Noise window** parameter is too low. Increase the **Noise window** parameter in the baseline calculation. This may in some cases result in peak limits too high up on the peak slopes (Optimising the baseline parameters using a classic algorithm for a description of **Noise window** with the classic algorithm).

Another possibility is to use the **Reject peaks** function in the **Integrate** dialogue to reduce the number of peaks based on an appropriate criterion, e.g. the number of peaks or the minimum peak height.

10.1.5 Optimising the baseline parameters using a classic algorithm

The first choice when trying to optimise the peak integration is to change the baseline parameters. For a brief description of the parameters and a suggested way of estimating settings for the baseline parameters from the source curve, see "Baseline calculation theory" on page 371. The **Integrate:Edit baseline** function may provide additional information about the cause of the unsatisfactory peak integration by revealing where the baseline points have been placed by the automatic baseline calculation algorithm.

When optimising the baseline calculation, a change in the **Slope limit** will very often give the expected result. An example of the effect of a too high **Slope limit** is that the up-slopes of the peaks may be recognised as baseline segments. Baselines that deviate substantially from the source curve may arise from a combination of a too long **Shortest baseline segment** value with a too high **Slope limit**.

A recommended strategy is to change the baseline parameters step by step and to check the resulting baseline after each change. The size of the initial change depends on the cause of the peak integration problem, but some general guidelines can be given. When the desired effect is achieved it is advisable to go back and check a parameter value in between the two last settings to avoid an unnecessarily low or high value. The default baseline parameters can be restored by clicking on the **Default** command button.

Baseline parameter	*Recommended initial change*
Shortest baseline segment	20-50%
Noise window	10-30%
Slope limit	25-50%
Max baseline level	Usually not necessary to adjust

Below are some examples of common problems with peak integration and baseline calculation, as well as suggestions for improvements. The following problems will be discussed:

- Baseline does not follow the source curve
- Peak limits too high up on the peaks
- Noise detected as peaks
- Peaks missing
- Baseline on top of peaks

Baseline slope does not follow the source curve

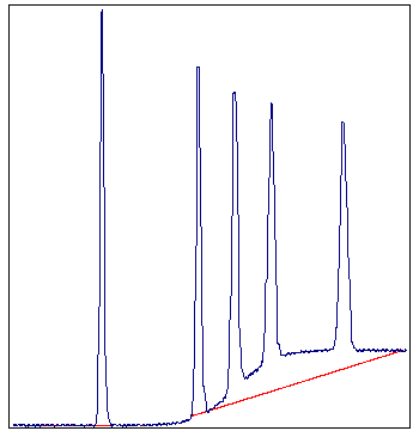


Fig 10-12. Baseline calculation with default parameters.

The calculated baseline does not follow the source curve, because short curve segments between peaks in the middle of the chromatogram are not identified as baseline segments. If you decrease the **Shortest baseline segment** by 50% the following baseline is calculated.

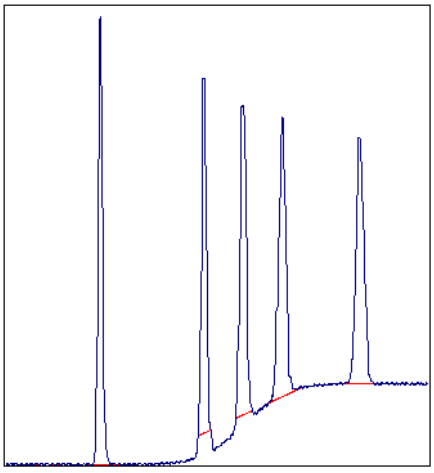


Fig 10-13. Baseline calculation with 50% lower Shortest baseline segment.

The baseline is, however, still unsatisfactory, due to the high slope of the short segments in the region between the second and fourth peak which are still not identified as baseline segments. If you increase the **Slope limit** by a factor 2.5, a correct baseline is calculated.

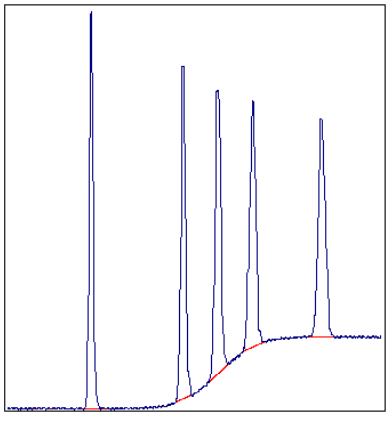


Fig 10-14. Correct baseline parameters.

Peak limits too high up on the peaks

This peak integration problem is in most cases caused by a too high value for the **Slope limit** and/or a too high value for the **Noise window.** This can be encountered when the chromatogram includes a very large

flow-through or solvent peak. The large peak affects the calculation of the default baseline parameters, leading to too high values for **Slope limit** and **Noise window**.

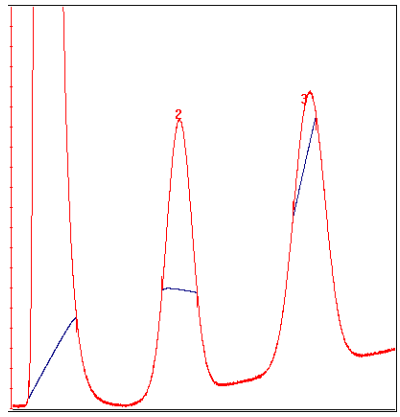


Fig 10-15. Peak start and end points on peaks caused by an excessively high Slope limit.

Note the difference between the situations in Figures 10-12 and 10-15. In Figure 10-12, no baseline segments were detected between the second and fourth peaks (there are no blue crosses is this region when **Edit baseline** is selected). The baseline follows the curve as a best fit, and cannot be drawn above the curve unless **Accept negative peaks** is selected. In Figure 10-15, baseline segments are detected on the up and down slopes of the peaks (marked by blue crosses in these regions when **Edit baseline** is selected). By considerably decreasing the **Slope limit** in Figure 10-16, a better baseline can be constructed, leading to an improved peak integration.

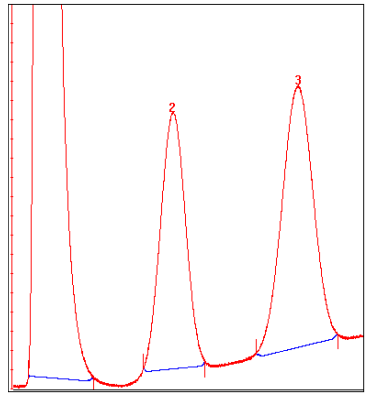


Fig 10-16. Peak integration with decreased Slope limit.

You may also have to decrease the **Noise window** to get a perfect peak integration.

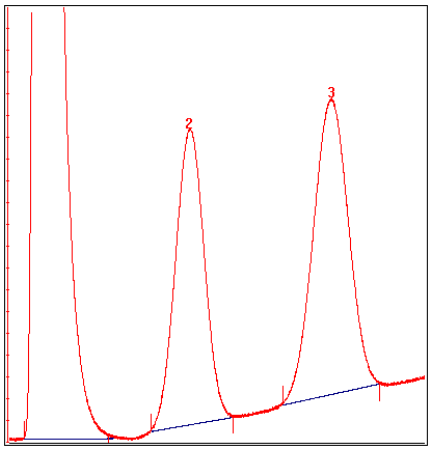


Fig 10-17. Correct baseline after decreasing both Slope limit and Noise window.

An alternative approach is to exclude the large peak from the peak integration, as its presence affects the default baseline parameters and the retention and area of the large peak is in most cases not interesting. Using the **Operations:Cut curve** function, the appropriate region of the chromatogram can be selected. The peak integration can then in most cases be performed with default baseline parameters on the cut curve.

Noise detected as peaks

Sometimes you get too many peaks after the peak integration, usually because noise on the baseline is erroneously detected as peaks.

The cause of this problem is that the **Noise window** parameter is too low. Increase the **Noise window** parameter in the baseline calculation. This may in some cases result in peak limits too high up on the peak slopes (see example below).

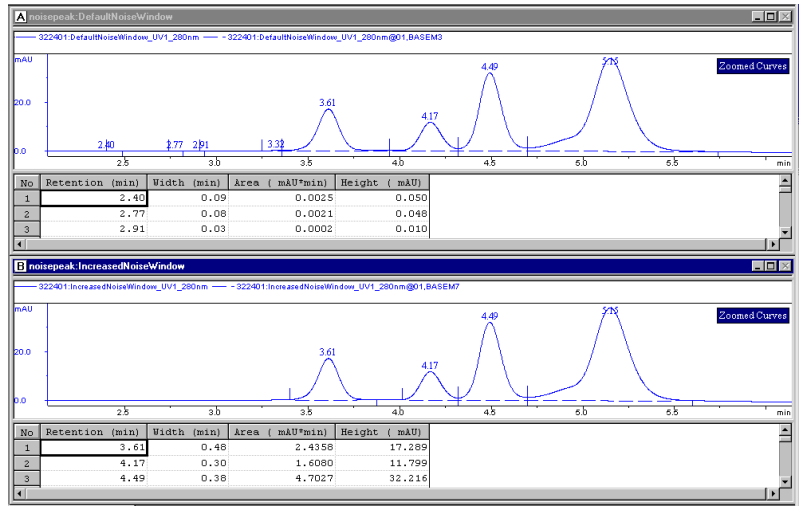


Fig 10-18. (A) Noise detected as peaks; (B) Peak integration after increase of Noise Window.

Another possibility is to use the **Reject peaks** function in the **Integrate** dialogue to reduce the number of peaks based on an appropriate criterion, e.g. the number of peaks or the minimum peak height.

Peaks missing

In cases where obvious peaks are not detected in the peak integration, a probable cause is that the **Noise window** parameter is too high.

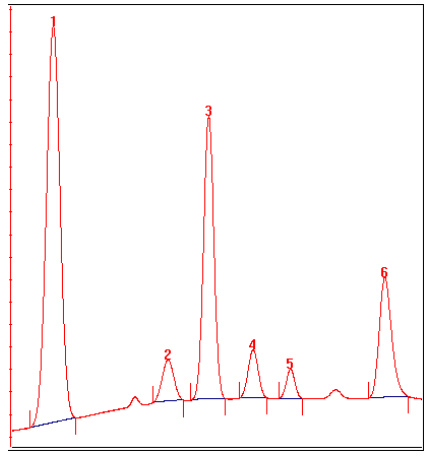


Fig 10-19. Peak integration with too high Noise window.

Decrease the **Noise window** parameter until the peaks are detected.

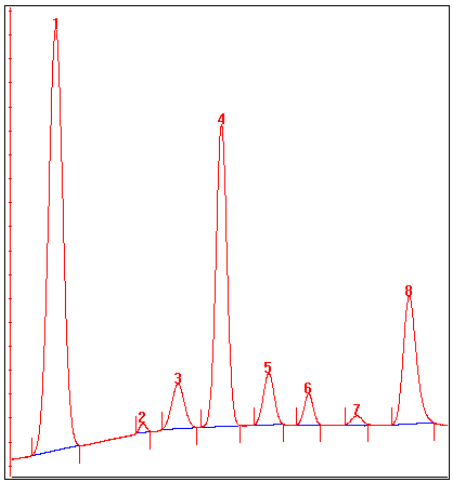


Fig 10-20. Correct peak integration after decreasing Noise window.

Another possible cause of missing peaks is that an improper reject criterion has been used. Check the criteria used for **Reject peaks** in the **Integrate** dialogue as well as **Filter** peaks in the **Chromatogram layout** function.

Baseline on top of peaks

In rare cases the top of a broad flat peak will be incorporated as a baseline segment.

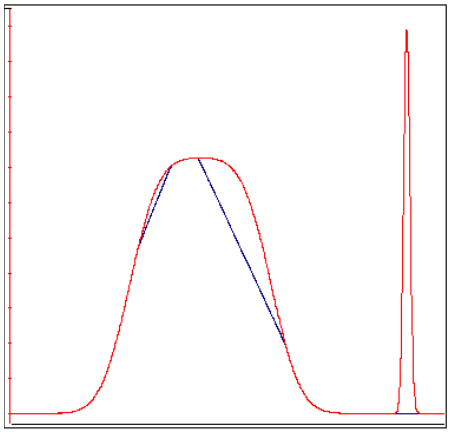


Fig 10-21. Baseline segment on top of peak.

This is one of the very few situations where it is useful to change the **Max baseline level**. Measure the height of the flat plateau of the peak using the **Marker** icon on the chromatogram (Measuring retention time and peak heights). Insert a value somewhat lower than the plateau height as the **Max baseline level** in the baseline calculation.

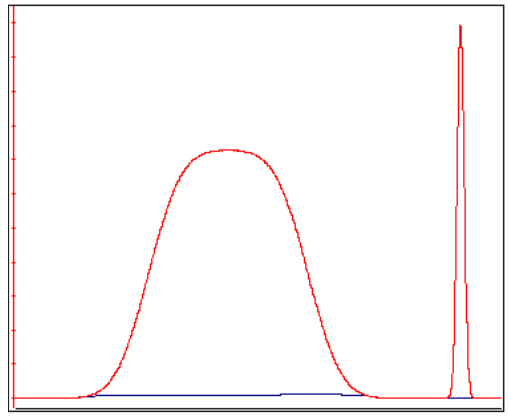


Fig 10-22. Correct baseline after decreasing Max baseline level.

If there are several rather short segments that erroneously have been incorporated in the baseline, an alternative remedy is to increase the **Shortest baseline segment** setting.

10.1.6 Manually editing a baseline

Once a baseline has been calculated, it is possible to add or remove baseline points on it and then draw a new baseline from the new set of data points. The edited baseline curve can then be used in a new peak integration.

1. Select **Integrate: Edit baseline**.

2. Choose the desired baseline from the displayed dialogue and click on **OK**. A window will appear displaying the baseline and the curve from which it was calculated. Additionally, blue crosses are displayed (the baseline points) and their co-ordinates in the **Point list**.

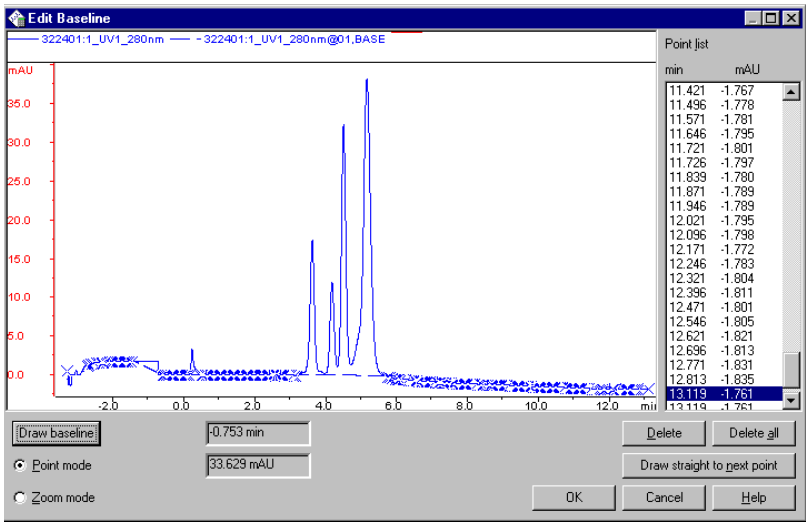


Fig 10-23. Edit Baseline chromatogram window.

Insertion of baseline data points

Select the **Point mode** button. Click on the left mouse button to place a new baseline point on the chromatogram. Each new point is represented by a cross and its co-ordinates are automatically entered into the **Point list**. This is useful when, for example, you want the baseline to go up to a high valley between two peaks.

To make your task easier you can click on **Zoom mode** and zoom in on specific regions of the chromatogram and then insert baseline data points. The shortcut menu allows you to undo the last zoom step with **Undo zoom** or to reset the default zoom scale with **Reset zoom**.

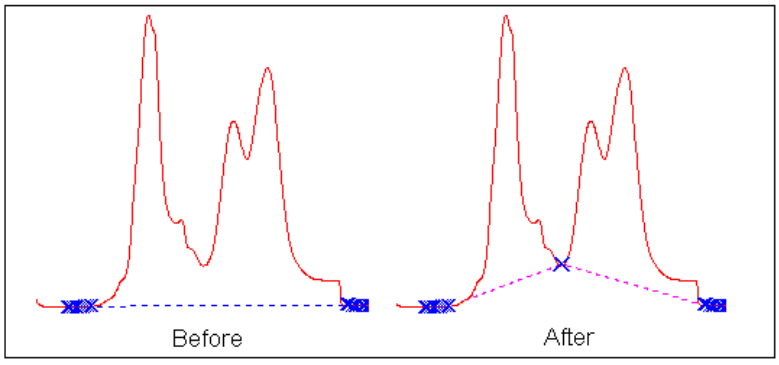


Fig 10-24. Baseline before and after editing.

Deleting baseline data points

If you want to delete a data point from the **Point list**, select the appropriate point in the list and press the **Delete** button. Alternatively, double click on the unwanted data point entry in the list to delete it. **Delete all** removes all baseline data points.

Drawing the new baseline

When you are satisfied with your baseline point selection, click on the **Draw baseline** button. The new baseline curve will be drawn as a spline function based on the previous and the new points. The spline function is guided by the points, but does not necessarily pass through them. You may also force a straight baseline between two points by selecting the first of the two points in the point list and then clicking on the **Draw straight to next point** button.

Click on **OK**, and the new baseline will be saved with the default name **Edited Baseline**. This may now be used as the baseline in a new peak integration.

10.1.7 Adjusting the peak limits

Once a peak table has been generated using the appropriate baseline, it is then possible to split or join peaks and to manually adjust the peak start and end points. The peaks will then be renumbered and the peak areas will be recalculated.

1. It is recommended that you first access **Curve Style and Colour** tab in the **Chromatogram Layout** dialogue, and select either **Number** or **Retention** for labelling the peaks. The former option will sequentially number each of the peaks in the chromatogram which is opened during the edit mode of a peak table. The latter option will display the retention volume or time for each peak.

2. Select **Integrate:Edit peak table**. Select the desired peak table from the displayed dialogue. Note that name of the baseline on which the selected peak table was based, is displayed at the bottom of the panel.

3. Double click on the desired peak table in the list or click on **OK**. A chromatogram window is displayed containing the selected peak table with corresponding curve and baseline. The various editing features are described below. As an aid, it is possible to use the zoom function.

4. Once you have completed your changes, click on **OK** and verify the destination of the new (edited) peak table.

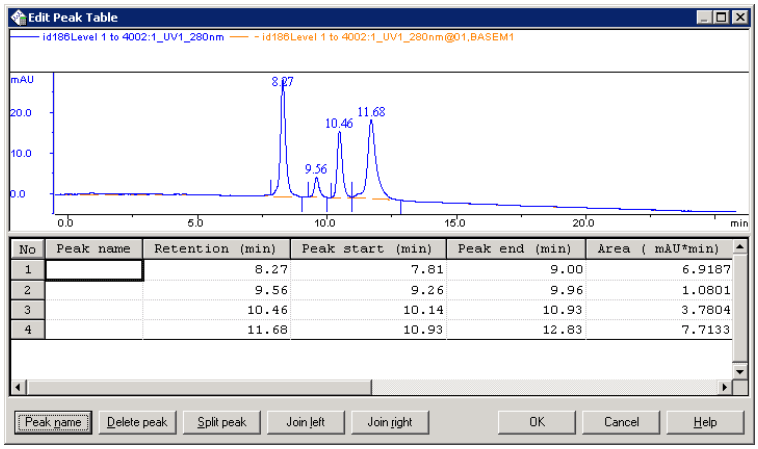


Fig 10-25. Edit Peak Table chromatogram window.

Deleting a peak in the peak table

To delete a peak from the table, click on the peak in the chromatogram or in the peak table and click on the **Delete peak** command button. Note that the remaining peaks will be renumbered after the deletion.

Splitting a peak

A peak is defined within delimiting drop-lines to the left and right of the peak. It is possible to split the peak into two new peaks by inserting a drop-line. The drop-line is always inserted at the middle point between two existing drop-lines. The area under each new peak will not be the same if the symmetry of the original peak was not perfect.

To make a split, select the desired peak in the list or mark in the curve and press the **Split Peak** button.

Note: The peaks will be renumbered according to the split. Refer to the description below about adjustment of the drop-lines.

Joining a peak

It is possible to join the areas of adjacent peaks if separated by a drop-line.

1. Select a peak either on the chromatogram or in the peak table.

2. Click on **Join left** or **Join right** if you want the peak to be joined with the peak to its left or right respectively. The original intervening drop-line is removed and all peaks are renumbered.

Adjusting peak start and end points

The beginning of each peak is marked with a drop-line above the curve, and the end of each peak is marked with a drop-line below the curve.

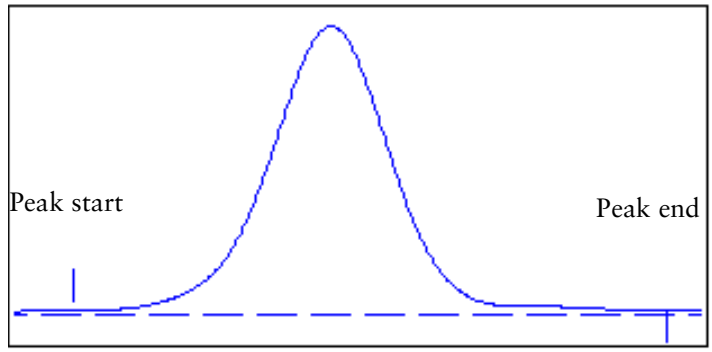


Fig 10-26. A drop-line at the start and end of a peak.

Where there are two peaks beside one another, the end of the first peak will be at the same point as the beginning of the next peak. Thus, there will be a drop-line below and above the line at the same point.

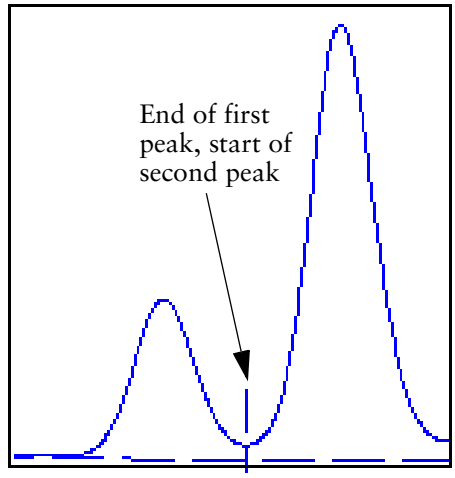


Fig 10-27. A drop-line between two peaks.

It is possible to move the drop-lines for a selected peak and thus affect the area beneath the peak.

1. Click on the peak of interest on the curve or in the peak table and two vertical cursor bars become superimposed on the left and right drop-lines that delimit the selected peak.

2. Drag the left and right drop-line bars to define the new left and right limits respectively for the selected peak. The drop-lines can never be moved beyond any other drop-line. The new left and right limits are now represented by a drop-line above and below the curve respectively, and the peak areas are automatically recalculated.

3. Drop-lines on preceding or following peaks can be similarly adjusted. Movement of these drop-lines can be up to, but never beyond, any other drop-line. A drop-line may also not be moved beyond a point where the peak meets the baseline.

Identification names for peaks

Double click on the peak of interest in the peak table spreadsheet cell and edit the name directly in the cell. You will see the peak names in the chromatogram (and the spreadsheet cell) only if you have earlier accessed **Curve Style and Colour** tab in the **Chromatogram Layout** dialogue, and selected **Peak name** for labelling the peaks. Note also that in order to have **Peak name** listed within the spreadsheet cell, you must choose the **Peak name** option under the **Peak Table** tab within the **Chromatogram Layout** dialogue.

10.1.8 Measuring retention time and peak heights

It is possible to determine the co-ordinates of any point on a curve and thus obtain values for retention and peak height. This is a useful tool for many other functions, such as for measuring the parameters used in baseline calculations. Co-ordinates can be obtained in two ways:

- Direct measurement
- Viewing peak table data

Direct measurement

1. From within the chromatogram area, display the shortcut menu and select **Marker**. A vertical line is displayed on the chromatogram and can be dragged left and right with the mouse pointer. The marker box (at the top left corner of the window) contains coloured text which shows the X-axis co-ordinate and Y-axis co-ordinate, both with their respective units. The colour and units of the Y-axis information corresponds to a specific curve of the same colour.

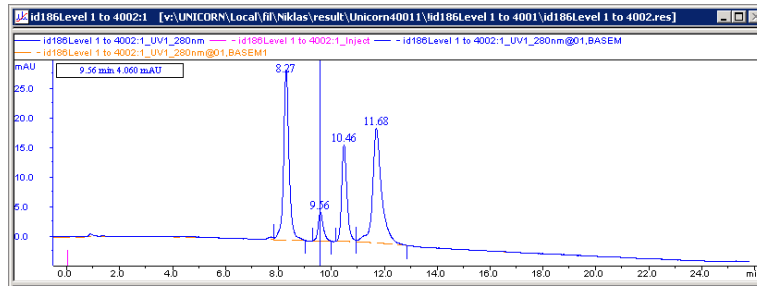


Fig 10-28. Active bar linked to a marker for determining curve point co-ordinates.

2. Click on the desired curve legend and the correct Y-axis is displayed.

3. To close this function, de-select from the shortcut menu.

Viewing peak table data

The retention time and amplitude of any peak can be directly viewed in a peak table after an integration, if selected for in the **Chromatogram Layout** dialogue.

10.1.9 Measuring HETP

HETP (height equivalent to a theoretical plate) calculations allow you to check how well the column has been packed.

1. Perform a run with injection of a non-interactive substance, for example, a small volume of acetone. Note that the injection must be at zero time.

2. In the **Integrate** dialogue, type in the column height (cm) in the **Column height** field. Perform the peak integration according to your other selected parameters.

3. To view the results of the integration, select **Plate height (HETP)** in the list within the **Peak Table** tab of the **Chromatogram Layout** dialogue.

Every peak will have a HETP value. A narrow peak gives a low value corresponding to a well packed column. A broad peak gives a high value, indicating a column that is not optimally packed.

HETP is calculated as follows:

HETP = L/N

N = 5.54 x $(V_R/w_h)^2$ assuming a Gaussian peak

where

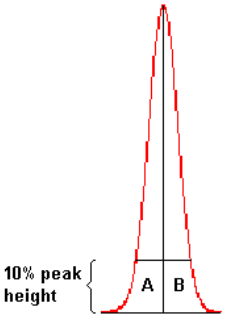
N = no. of theoretical plates

L = bed height in cm

V_R = peak retention (elution) volume or time

w_h = peak width at half height expressed in the same units as VR

10.1.10 Measuring peak asymmetry



This function can be used in combination with HETP to help assess column performance. A perfect peak will have no asymmetry and, after peak integration, give a value of 1.0. Any value less than 1.0 means that there is a left skew, i.e. the asymmetry falls on the leading side (left) of the peak. The reverse is true for values greater than 1.0 where the asymmetry comes on the tailing side (right) of the peak.

To view the asymmetry data select **Asymmetry** in the list within the **Peak Table** tab of the **Chromatogram Layout** dialogue. Click on **OK** and return to the chromatogram window.

Asymmetry = width B / width A, where A and B are the partial peak widths measured at 10% of the peak height, with A representing the leading part of the peak and B the tailing part of the peak.

10.1.11 Measuring resolution

Resolution is a measure of the relative separation between two peaks and can be used to determine if further optimisation of the chromatographic procedure is necessary. If the resolution value is 1.0, then 98% purity has been achieved at 98% of peak recovery, provided the peaks are Gaussian and approximately equal in size. Baseline resolution requires that the resolution value is greater than or equal to 1.5. At this resolution, purity of the peak is 100%.

To view the resolution data, select **Resolution** in the list within the **Peak Table** tab of the **Chromatogram Layout** dialogue. Click on **OK** and return to the chromatogram window. The resolution value for each peak shows the resolution with respect to the previous peak.

$$R = \frac{(V_{R2} - V_{R1}) \times 1.177}{w_{h1} + w_{h2}}$$

where:

$V_{R2} > V_{R1}$

V_{R1} = retention (elution) volume for peak 1

V_{R2} = retention (elution) volume for peak 2

w_{h1} = peak width at half height for peak 1 (for Gaussian peaks)

w_{h2} = peak width at half height for peak 2 (for Gaussian peaks)

See Appendix Section D-3 for alternative calculations of the resolution.

10.2 Other evaluations

10.2.1 Peak purity and peak identification

Ratios between UV curves measured at different wavelengths give useful information about peak purity or peak identity. The **Operations:Divide** function can be used when you have a result file with run detected at more than one wavelength.

1. Before dividing the curves, you must make sure that both curves have a baseline close to zero AU. This can be achieved with baseline subtraction.

2. Create a baseline for each UV curve according to the procedures detailed in Sections 10.1.1 and 10.1.2. Subtract the baselines from their respective UV curves using **Operations:Subtract**.

   Alternatively, you can subtract the corresponding blank runs from the UV curves if such exist.

3. When you have achieved two curves with a zero baseline, select **Operations:Divide** and select the two curves for division. You have the option to set **Threshold** values by checking the option and setting the threshold value for each curve. This sets the quotient to 1.0 if either of the sample values is closer to zero than the threshold value. This is to prevent very high quotient values being created if division is performed with values close to zero. Very low quotient values are also prevented. **Threshold** values are suggested by UNICORN although these can be changed.

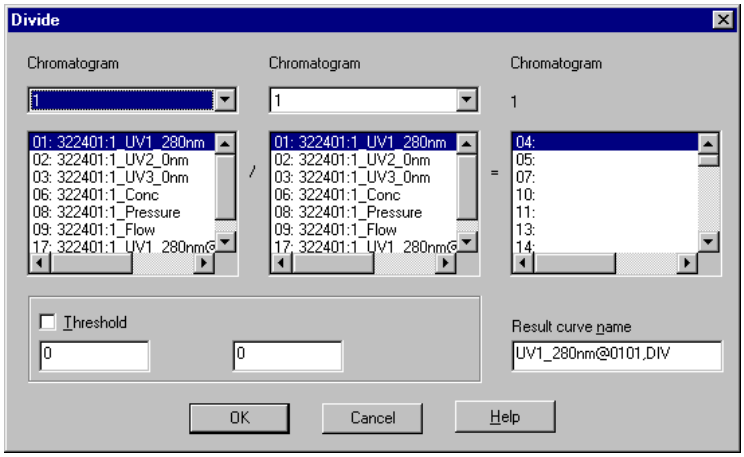


Fig 10-29. Divide dialogue.

4. The resulting curve can then be filtered by **Operations:Smooth** (Reducing noise and removing ghost peaks). It is suggested that you smooth using the median filter to remove noise that appears as spikes or occurs in a small area of the curve.

The ratio can be used to check peak purity. If the peak is pure, the absorbance spectra are the same over the whole peak and therefore the ratios should remain constant. If the absorbance ratio is not the same over the whole peak, then the peak is probably not pure.

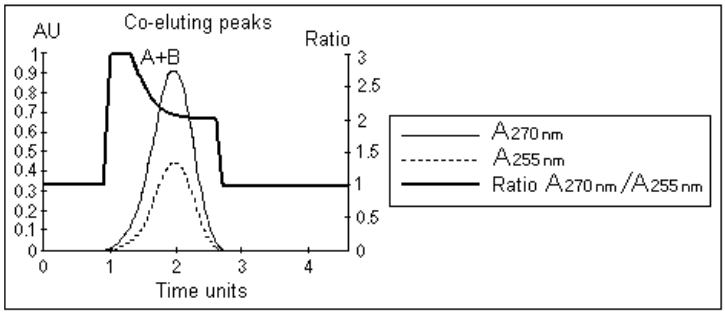


Fig 10-30. Simulated chromatogram of two co-eluting components with differing absorbance spectra and a small difference in retention time.

The resulting ratio can also be used for peak identification as different compounds have a specific ratio between absorbencies at different wavelengths.

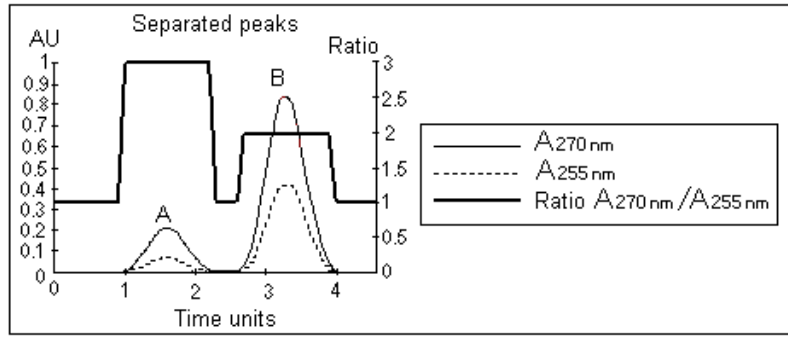


Fig 10-31. Simulated chromatogram of two components with differences in their absorbance spectra.

10.2.2 Simulate Peak Fractionation

To create a curve that simulates a peak fractionation select **Operations:Simulate Peak Fractionation.**

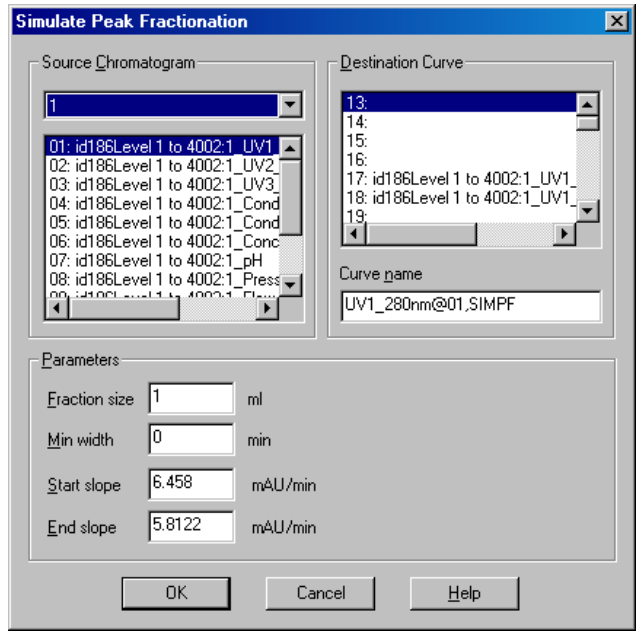


Fig 10-32. The Simulate Peak Fractionation dialogue.

1. Select the **Source Chromatogram** and thereafter the curve for which a simulated peak fractionation is to generated.

2. Select the **Destination Curve**.

3. Enter a **Curve name** if other than the suggested name.

4. Enter values for **Parameters; Fraction size, Min width, Start slope** and / or **End slope.**

5. Click on **OK.**

The simulated peak fraction curve is displayed on the chromatogram and alignment of the fraction label text can be changed in the **Chromatogram Layout** box.

10.2.3 Finding the slope values for Peak Fractionation or Watch instructions

With ÄKTAdesign systems it is possible to only collect peaks during fractionation. Peak fractionation parameters are set in the Method Editor with the instruction **Peak_FracParameters**. **StartSlope** and **EndSlope** values are set. The procedures for finding suitable slope values for a particular run are described below.

It is also possible to set up conditional (**Watch**) instructions which allow the progress of a run to be determined by the events during the run, e.g. start collecting fractions when the first peak emerges. The slope of the curve may be set as a condition used to satisfy a **Watch** condition in the method during the run. It is therefore important to use accurate slope values for the specific **Watch** instruction parameter.

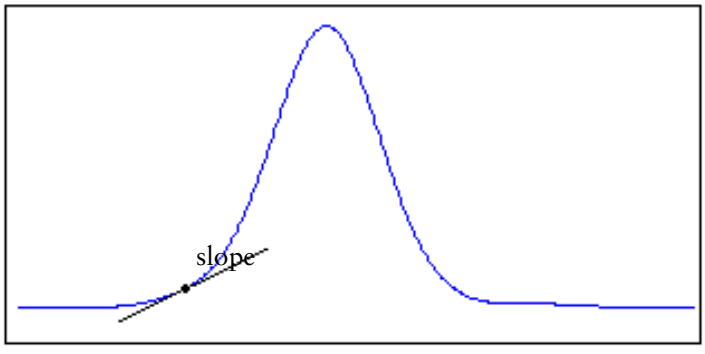


Fig 10-33. The slope of a curve.

To determine slope values, you must first make a run with the sample you intend to purify. Then use this result to find slope values in the Evaluation module:

1. Ensure that you have selected **Time** as the X-axis scale for retention in the **Chromatogram Layout** dialogue, **X-Axis** tab.

2. Select **Operations:Differentiate**. Select the desired (UV) curve, check that a **First order** calculation is selected and click on **OK**. The differentiated curve will appear in the active chromatogram.

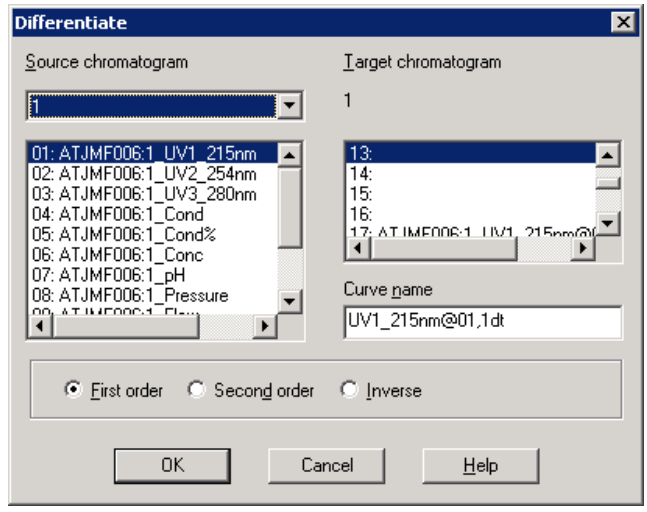


Fig 10-34. Differentiate dialogue.

3. Measure the Y-axis values on the differentiated curve by selecting **Marker** from the shortcut menu, choosing the Y-axis differentiate scale and reading the curve co-ordinates in the active **Marker** box. It may be necessary to smooth the differentiated curve. The units for the differentiated curve is mAU/min or AU/min. Co-ordinates are based upon the position of the vertical line on the chromatogram in relation to where it bisects the curve. Any Y-axis value for the differentiated curve is the UV curve slope at the selected retention point.

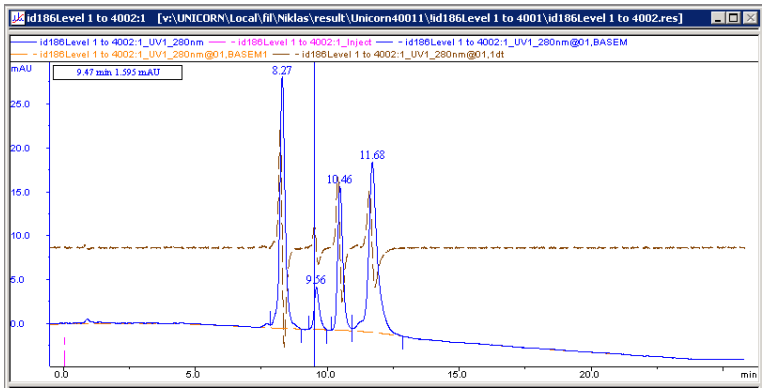


Fig 10-35. Measurement of the Slope limit after differentiation.

4. Use the zoom function to magnify the curve over an appropriate area. Place the vertical cursor bar at the beginning of a peak where you want the **Watch** conditions to be fulfilled, i.e. where the slope becomes higher. Read the actual slope value in the active **Marker** box.

   When using the **PeakFractionation** instruction for ÄKTAdesign systems, measure the slope at the beginning and end of the smallest flattest peak to collect and use these values.

5. In the Method Editor, enter the slope value as a parameter for the Watch instruction or enter the **StartSlope** and **EndSlope** values in the **Peak_FracParameters** instruction.

10.2.4 Creating a curve

It is possible to create a curve based on any external measurements.

1. Select **Operations:Create Curve**. In the dialogue that is displayed, select one or several help curve(s).

2. Select the minimum and maximum values of the Y-axis. Also choose the appropriate units from the **Unit** list. The help curve determines the min and max values for the X-axis.

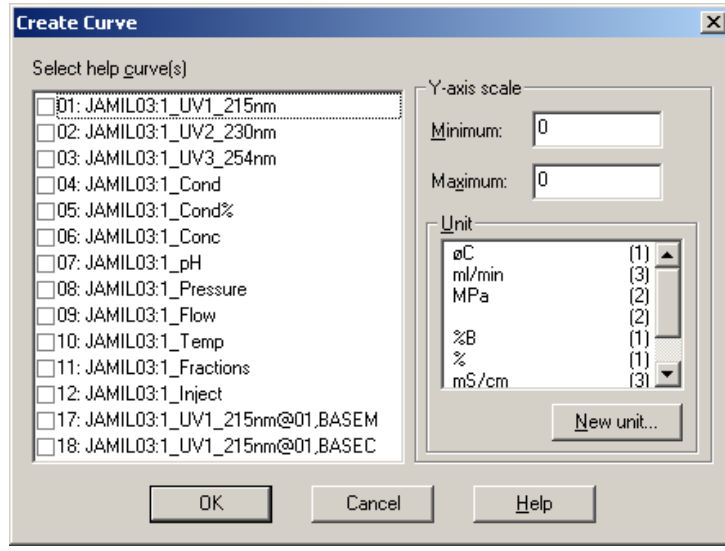


Fig 10-36. Create Curve dialogue.

3. If you want to create new unit, click on the **New unit** button and enter the new unit name and number of decimal places.

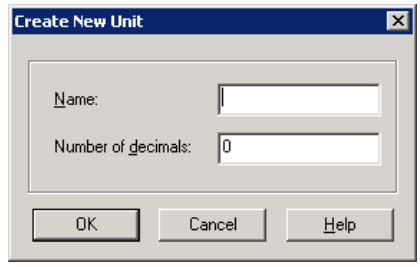


Fig 10-37. Create New Unit dialogue.

Click on **OK** to return to the **Create Curve** dialogue and again on **OK** when you have made your selections there.

4. With **Point Mode** selected, you can use the left button to insert new curve points on the chromatogram. The co-ordinates of each new point are automatically entered into the **Point list**.

The co-ordinates of the mouse cursor are displayed beneath the curves thus allowing you to precisely position a new data point. The co-ordinates for the new curve are always displayed. Selecting **Zoom mode** allows you to use the mouse to select an area of the view and zoom in. The shortcut menu allows you to undo the last zoom step with **Undo zoom** or to reset the default zoom scale with **Reset zoom**. In **Zoom mode** you can also drag the vertical cursor line and the co-ordinates presented reflect where the line bisects the curve of the same colour.

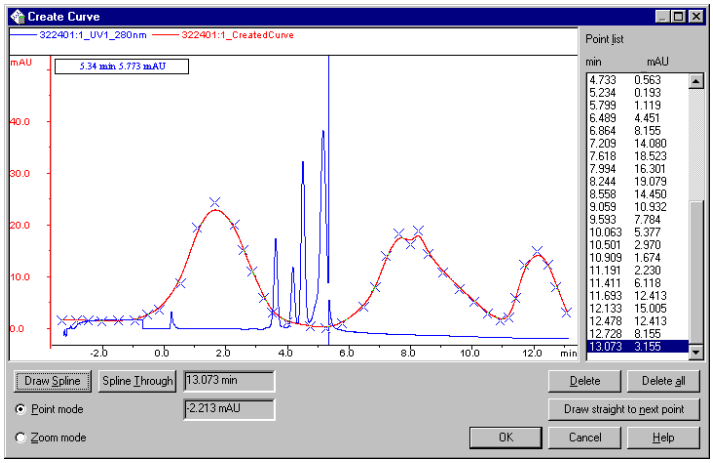


Fig 10-38. Create Curve chromatogram window.

5. To delete a point from the **Point list**, double click on the appropriate choice in the list. Alternatively, select the co-ordinates in the list and click on the **Delete** button. To delete all of the points in the list, click on the **Delete All** button.

6. To draw the curve, click on either **Draw spline** or **Spline through**. **Draw spline** creates a smooth curve from the data but does not necessarily pass through every point that you have entered. By contrast, **Spline through** creates a curve that passes through all of the data points.

7. In cases where you have created a curve using **Draw Spline**, you may want the curve to pass through a selection of those points currently lying away from the curve. You may force a straight line between two points by selecting the first of the two points in the **Point list** and then clicking on the **Draw straight to next point** button. This may have to be repeated for several consecutive points to achieve the desired curve.

8. Click on **OK** and save the curve. You can change the curve name from the default, **CreatedCurve**, and also the curve destination.

10.2.5 Measuring salt concentrations in the fractions

If you need to know the approximate conductivity or concentration of salt in your fractions, it is possible to calculate these from the conductivity curve.

Note: The conductivity signal is not linear above 0.3 M, but you will still gain a relatively good idea of the salt concentrations above 0.3 M.

A conductivity curve, usually given the name **Cond**, is stored in a chromatogram within a result file. This curve represents the real conductivity data in mS/cm and should be used for calculations. Another curve, **Cond%**, is also present and is the same as **Cond** but re-scaled to display percentage values.

To make the calculations:

1. Select **Operations:Fraction histogram**. Select the **Cond** curve in the left list and the fractions curve should already be selected in the middle list. If you have earlier pooled fractions, it is possible to select the desired fraction curve. Click on **OK**.

2. Select the appropriate fraction curve in the **Chromatogram Layout** dialogue. In the active chromatogram you will see the fraction marks, the fraction histogram of the conductivity curve and any other selected curves. Select **Marker** from the shortcut menu to display the vertical cursor line. Click on the desired curve legend and the corresponding Y-axis is displayed.

3. Use the mouse to drag the vertical bar back and forth along the X-axis. For a given fraction, its conductivity is displayed in the marker box.

10.3 Automated evaluation procedures

An evaluation procedure is a recorded sequence of interactive operations in the Evaluation module, which can be executed for automated data evaluation and report generation. It can be used for single chromatograms and for a number of chromatograms in different result files. The concept is analogous to the "macro" facility provided in many word processing and other programs. Evaluation procedures can also be called from methods, making run execution, evaluation and documentation fully automatic. Automation is achieved using the **Procedures** menu.

A procedure can be recorded and run using the **Procedures** menu commands or from the commands available in the **Procedure Editor** dialogue. The **Procedure Editor** dialogue also allows you to view and edit the instructions within a procedure.

10.3.1 Recording a procedure

1. Open the appropriate results file in the Evaluation module.

2. To begin recording a procedure select **Procedures:Record on**. The **Procedure Editor** dialogue is displayed in Record mode.

3. Minimize the **Procedure Editor** dialogue.

4. Perform the evaluation steps that the procedure is to contain. These steps are recorded as you perform them.

5. To stop the recording, either:

   - select **Procedures:Record off**, or
   - restore the iconised **Procedure Editor** dialogue and click on the '**stop**' button or select the dialogue **Control:End Record** menu command.

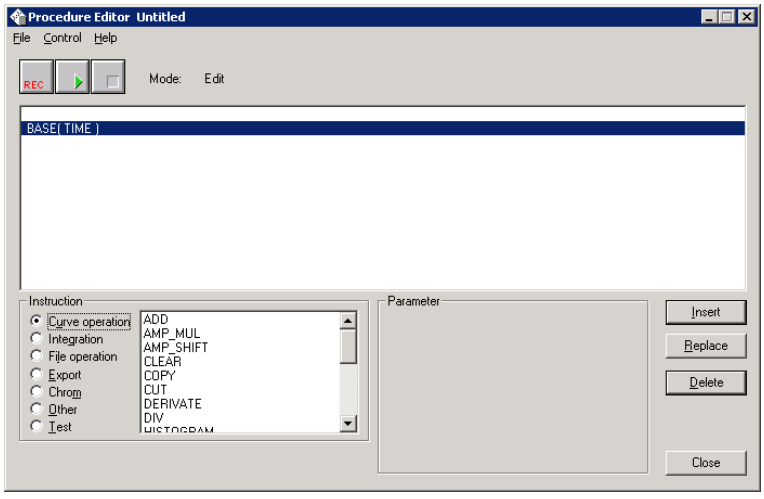


Fig 10-39. Procedure Editor dialogue.

6. The recorded procedure can be viewed in the **Procedure Editor** dialogue. Restore the iconised dialogue if necessary.

7. More steps can be added to the evaluation procedure by repeating steps 2-6. The new steps are added to the previous procedure.

Note: New lines will be inserted into the procedure after the selected line in the currently listed procedure. This can be used to insert new instructions between existing instructions.

8. If required edit the evaluation procedure (Editing an existing procedure).

9. Select **File:Save** or **File:Save as** from the dialogue menu and enter a name for the procedure. The evaluation procedure is saved within UNICORN and is specific to your user name. If you have **Edit global list(s)** access you may also check the **Global procedure** option to make the procedure available to all users. Global procedures are marked with **(Global)** before the name. Even if the results of an evaluation session are not saved, the created evaluation procedure(s) are saved.

10. Choose dialogue **File:Exit** menu command.

Note: If you already have an existing procedure open for editing in the **Procedure Editor** dialogue (Editing an existing procedure) and you follow the above procedure, new instructions will be added to the currently open procedure, i.e. you will not be creating a new procedure.

To create a new procedure, select **File:New:Procedure** or **Procedure:Edit:New** to display the **Procedure Editor** dialogue. To begin recording, click on the **Rec** button or select the dialogue **Control:Record** menu command.

10.3.2 Editing an existing procedure

Evaluation operations are represented by instructions in the **Procedure Editor** dialogue. These may be modified to suit specific evaluation needs and be saved for later use (see "Evaluation functions and instructions" on page 369).

Note: You are recommended to be restrictive in editing existing global procedures. It is recommended to open an existing procedure and save a copy under a new name and use this copy to perform any editing procedures.

1. Click on **Procedures:Edit:Open** and select the desired evaluation procedure from the list. The **Procedure Editor** dialogue is displayed for the selected procedure.

2. To view the parameters contained within a specific instruction in the procedure, select it and view the information contained within the **Instructions** and **Parameters** fields of the dialogue.

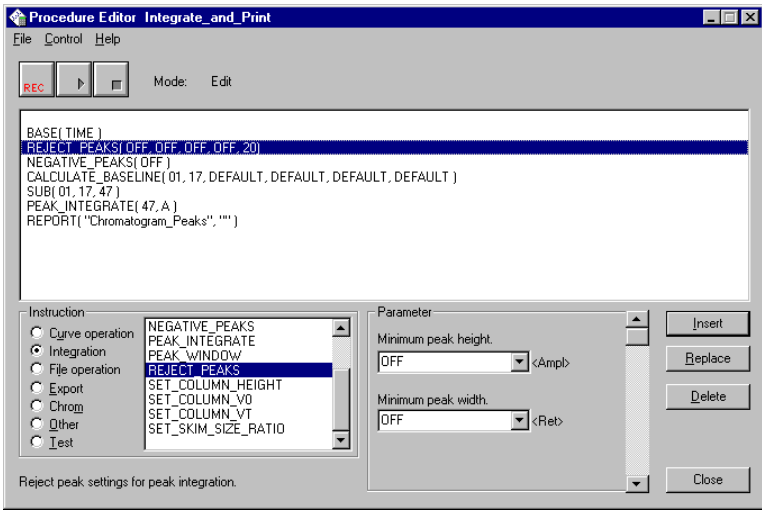


Fig 10-40. Editing procedures in the Procedure editor dialogue.

There are several types of instructions, as denoted by the options buttons, e.g. **Curve operation**, **Integration**, **File operation** etc., in the **Instructions** field. The appropriate option button and instruction therein will be automatically selected when you select an instruction in the procedure.

The specific parameters contained within the selected procedure instruction are displayed in the **Parameters** field.

A simple definition of the selected instruction is displayed at the bottom left-hand corner of the dialogue. You can also select an instruction and then press F1, or select **Help:Index:Instruction**. For a list of procedure instructions with fuller descriptions and parameters see “Evaluation procedure” on page 380.

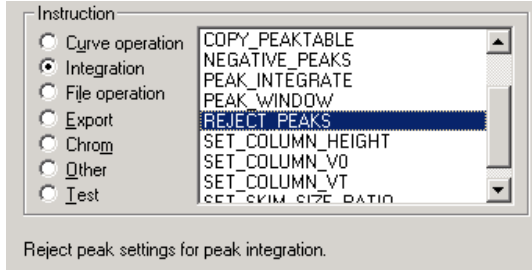


Fig 10-41. Instruction field in the Procedure editor dialogue.

3. To edit the parameters of a selected instruction, enter the new value in the appropriate place in the **Parameters** field. When you have made the desired changes, click on the **Replace** command button. You will now see that the selected instruction in the evaluation procedure is updated in accordance with the new parameters assigned to it.

4. You can insert new instructions *after* the currently selected procedure instruction. Select an instruction type and instruction in the **Instructions** field and enter the appropriate parameter values in the **Parameters** field. Click on the **Insert** command button. The new instruction will now be added to the evaluation procedure.

5. To remove an instruction from the evaluation procedure, select it in the procedure listing and click on the **Delete** button.

6. To save the edited procedure select **File:Save** or **File:Save as** from the dialogue menu commands. Name the procedure.

7. Choose **File:Exit** from the menu bar in the dialogue.

10.3.3 Renaming and removing procedures

Procedures that you have created can be renamed or deleted from the list of available procedures.

Note: It is recommended that you exercise caution in renaming and removing existing global procedures.

Renaming a procedure

1. Select **Procedures:Edit:Rename**.
2. Select a procedure in the **Rename Procedure** dialogue.
3. Click on **OK**.

Removing a procedure

1. Select **Procedures:Edit:Delete.**
2. Select a procedure in the **Delete Procedure** dialogue.
3. Click on **OK**.

10.3.4 Points to watch

In recording and editing evaluation procedures for automatic evaluation, beware of the following potential pitfalls:

- Make sure that the procedure addresses the right curves. Curves are identified by storage position alone: thus the instruction ADD (01,02,03) will try to add curve 01 to curve 02 and store the result in 03, regardless of the contents of 01 and 02. If 03 contains a curve which is not a raw data curve, the existing curve in 03 will be overwritten. If 03 contains a raw data curve, the procedure will stop with an error message. The raw data curves will always occupy the same positions for a given strategy, e.g. UV in position 01. If the operation is not valid when the procedure is run, the procedure will stop at the instruction with an error message. Any subsequent instructions in the procedure will not be executed.
- In calculating a baseline using the "classic" algorithm, UNICORN suggests default values for the four control parameters (Optimising the baseline parameters using a classic algorithm) based on the appearance of the curve. To instruct UNICORN to use default values appropriate for the curve every time the procedure is run, choose the Default setting in the appropriate fields for the parameters.

  For example:

  CALCULATE_BASELINE (01, 06, XXX, XXX, XXX, XXX)

  can be changed to:

  CALCULATE_BASELINE (01, 06, DEFAULT, DEFAULT, DEFAULT, DEFAULT)

10.3.5 Running evaluation procedures

To run a procedure for a specific chromatogram, first make sure that the desired chromatogram is active. Click on **Procedures:Run** and choose the desired evaluation procedure. Click on **OK** and the procedure runs at once. You can also open the **Procedure Editor** dialogue and select the dialogue **Control:Run** menu command or click on the 'Play' button.

10.3.6 Batch runs

It is possible to apply an evaluation procedure to a designated batch of result files even if they are not open on the Evaluation workspace. It is especially useful, for example, to perform integration with the same parameter settings on many results, or to print a number of results with the same settings. The batch run is done in the background of the Evaluation module and thus the results of the run are not seen. You will, of course, receive any print-outs or report documentation if this was one of the steps in the run procedure (Run documentation).

1. Select **Procedures:Batch run** and the **Open Procedure** dialogue is displayed. Select the evaluation procedure and click on **OK**. The **Batch Run** dialogue, which allows you to search for the result files and/or chromatograms on which you wish to perform the batch run.

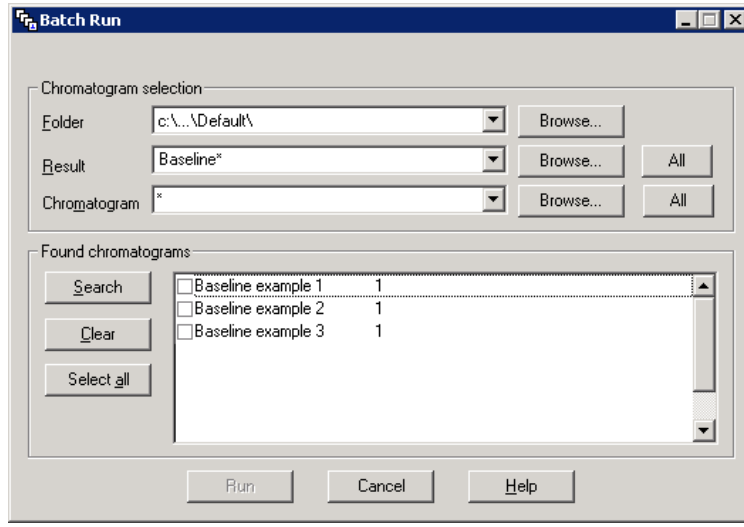


Fig 10-42. Batch run dialogue.

2. The search will take place in the displayed folder only. To select another folder, click on the **Browse** button and open the desired folder.

3. By default, all chromatograms are searched for within the designated folder and result files, as denoted by the asterisk '*****'. As for result files you can select a specific result file using the **Browse** function or you can use wildcard characters to search for chromatograms with a specific name profile.

You can use standard wildcard characters in the file name specification (* stands for any number of characters and ? for any single character). For example:

iex	will search files named iex
iex*	will search all files with names beginning iex
*iex	will search all files with names ending iex
?iex	will search only 4-character names ending in iex

User-entered search filters (up to a maximum of 10) will be saved in the drop-down menus for both **Result** and **Chromatogram** selections. More than one string can be used as a search delimiter (by inserting a ';' between strings). Search filters are automatically saved and stored within user profiles.

To return to the default setting to search for all chromatograms, click on **All**.

4. Click on the **Search** button and a list of chromatograms will be displayed based on the designated search criteria.

5. Select the chromatograms that you want to import. If you click on the **Select All** button, all of the displayed chromatograms are selected. If you want to clear the list of displayed chromatograms, click on **Clear**.

6. Click on **Run** to perform the batch run. Any created curves and peak tables will be saved in each result file automatically.

7. To view the results of a batch run on a specific result/ chromatogram, open this in the Evaluation workspace.

10.3.7 Evaluation procedures and reports

The creation of evaluation procedures, combined with batch runs, is a very useful tool to produce printed documentation simultaneously for many result files, e.g. for a number of scouting runs. This removes the necessity to open/import result files onto the Evaluation workspace.

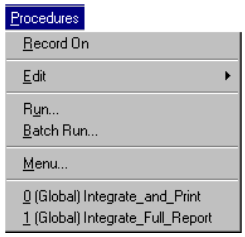
1. Begin recording a new procedure by selecting **Procedures:Record on**.

2. Select **File:Report** and choose a report format (Run documentation).

3. Select **Print** in the **Generate Report** dialogue as the final instruction.
4. Stop the record function by selecting **Procedures:Record Off**.
5. Save the procedure.
6. Now do a batch run (Batch runs) on all the desired result files to get the printed reports. The procedure can also be saved with a method to get automatic printouts at the end of a run.

Note: If the selected report format is changed in **File:Report** the new format will be applied when the procedure is run (except in cases where the procedure has been imported to a method - in these cases, the procedure is saved in the method file and cannot be changed). If the format is subsequently deleted, the procedure cannot be run.

10.3.8 Placing a procedure on the menu and running



It is possible to choose a maximum of 15 created evaluation procedures to be placed onto the **Procedures** menu.

Select **Procedures:Menu** and select the evaluation procedure to be added to the menu. Click on **OK**. Activate a chromatogram and select the **Procedures** menu. You will see the procedure that you added to the menu. Select this procedure and it is automatically run for the active chromatogram. The menu addition is remembered in UNICORN even if the results of the current evaluation session are not saved. To take away a procedure from the menu, deselect it from the **Procedures:Menu** list.

10.3.9 Exporting data or curves

Data and curves can be exported to other file formats. Select **File:Export** and then the appropriate cascade menu choice. Alternatively, the **Edit:Copy to clipboard** function can be used, which is the quickest and easiest way to copy a chromatogram into, for example, a word processing program.

Data can be exported to the following formats:

- ASCII (.asc)
- Lotus 1-2-3 (.wks or .xls)
- XML (.xml)
- AIA (.cdf)

Select the format that best matches your other application software. Peak tables are exported as text strings in the ASCII format, but as numerical values in the Lotus 1-2-3 (.wks or .xls) format. When exporting peak tables, all possible columns in the table are exported.

Curves are exported as series of numerical co-ordinates referring to time/volume and signal respectively. Multiple curves can be exported in each file. When exporting a curve, you may choose to export only a portion of the curve by inserting the limiting retention values directly into the boxes in the **Cut curve** field or by visually selecting the part of the curve using the **Cut graphically** option. To optimise the size of exported files, you may want to reduce the number of data points that are exported by pressing the spin buttons in the **Reduce number of samples** box to change the **Max no. samples** and the **Reduce by factor** function. The **Reduce by factor** function decreases the frequency with which data points are sampled for export. For example, reducing the number of data points by a factor of five will export every fifth point to the file. By checking the **Normalise retention** button all curves to be exported are normalised to a common X-axis.

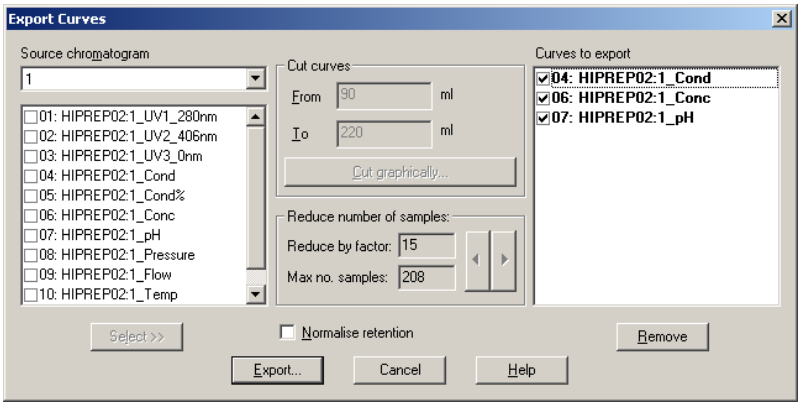


Fig 10-43. Export Curve dialogue.

Pressing the **Export** button will then prompt a dialogue where you can choose the new file name and destination. The default name is the same as the name of the current file. The extension of the exported file will be .asc, .cdf, .wks, or .xls, depending on the file format chosen.

10.3.10 Copying results to the clipboard

A related activity is **Edit:Copy to Clipboard** where the contents of the active window are copied to the Windows clipboard and can be later easily pasted into other programs, e.g. Microsoft®Word™. Curves and Documentation are copied as Windows enhanced metafiles (.emf), while Peak tables are copied as text. In the latter case, only the columns selected in the spreadsheet will be copied.

10.3.11 Importing results and curves

You may import curves in AIA (.cdf), ASCII (text) and Lotus 1-2-3 (.wks or .xls) spreadsheet formats, and access results from SMART Manager FPLCdirector and PrimeView. A wizard guides the user through the operation if PrimeView is chosen.

Note: When importing SMART or FPLCdirector files from a diskette it is recommended that you first copy files to the hard disc and then import the files.

Select **File:Import** and then the appropriate menu choice. A new dialogue will then prompt you to choose the source folder and file. The imported curve or result file is automatically opened to the screen.

11 Analysing results

Note: The procedures outlined in this chapter refer to the Analysis module, an optional UNICORN feature that must be ordered and installed.

11.1 Introduction

This chapter describes how to extend the UNICORN Evaluation module with an optional Analysis module to:

- determine the absolute quantity or concentration of a component
- determine the molecular size of a component

The Analysis module is accessed in the **Evaluation** window under two new menus, **Quantitate** and **Mol. Size**. The Analysis module uses many of the facilities within **Evaluation** and it is assumed that the user is familiar with these (see Chapters 9 and 10).

11.1.1 Quantitate

Quantitate extends the facilities in the Evaluation module to provide a wide range of techniques for quantitative analysis:

- External standard quantitation
- Internal standard quantitation
- Standard addition
- Recovery calculations.

Quantitate uses peak data from standard runs to produce calibration curves which can then be used to evaluate the amount and concentration of components in a sample.

Preparation of calibration curves is described in section 11.3. Calculating amount and concentration is described in Section 11.4.

11.1.2 Molecular size

The Molecular size function is used to determine the molecular size of components in a sample using a molecular size curve prepared from one or more standards.

Molecular size calculations are described in Section 11.6.

11.1.3 Definitions

Standard	A defined concentration of one or several components. The concentration does not need to be the same for all components in the standard. One or several standards are used to produce a calibration curve. For molecular size calculations the standard contains components of known molecular size.
Sample	A sample with an unknown concentration of the component(s) of interest. The concentration is determined by Quantitation. For molecular size calculations the sample contains components of unknown molecular size.
Standard run	A chromatographic standard run of a specific concentration level of a standard.
Sample run	A chromatographic sample run of a sample to be analysed.
Amount	This means injected amount. In most cases in the Manual "amount" is used as short for "concentration or amount". Each of them can be used to produce the calibration curve. When analysing the sample, both amount and concentration are calculated.
Spiking	The addition of a known quantity of the component of interest to the sample prior to the sample preparation for the run.
Peak size	Used throughout the manual as a common term for "peak area or peak height".
Peak table	The result of a peak integration presented in tabular form. The peak table can include, for example, height, area, and retention volume. After the analysis, the peak table contains the values for concentration, amount (and molecular size).
Calibration curve	The relationship between amount and peak size of a component. The relationship can be shown as a curve and as a mathematical expression.

Quantitation table	All necessary data required to quantitate one or several components in a sample. The Quantitation table contains calibration curve(s) and peak identification settings.
Level	A known amount or concentration of a standard. The Levels are numbered 1-20 in decreasing order of concentration.
Molecular size curve	The relationship between molecular size and retention volume for a number of components. The relationship can be shown as a curve and as a mathematical expression.
Molecular size table	All necessary data required to determine the molecular size of one or several components in a sample. The Molecular size table contains the molecular size curve.

11.1.4 Installation

If you are using a network, UNICORN 4.0 must be present on all the computers but this is not the case for the Analysis module which can be installed on just one or on several of the computers.

See the licence agreement for information on legal aspects of the installation.

Install the Analysis module as follows:

1. Insert the diskette in floppy (A:).
2. From the Desktop, close all applications on the computer.
3. Open **My Computer** and double click the **3½ floppy(A:)** icon.
4. Double click **Setup.exe** in the floppy drive file window to run the installation program. Follow the instructions on the screen.
5. When installation is complete, remove the diskette and restart the computer.

11.2 Quantitation overview

11.2.1 About quantitation

Quantitation is used to determine the amount or concentration of components in a sample. This chapter gives an overview over four

quantitation techniques provided by the Analysis module in UNICORN.

Quantitation should only be performed on chromatograms that have been saved in the current result file(s).

General points

Most quantitation techniques use peak integration data from standards to produce calibration curves. These curves show the relationship between the amount of the components of interest and the peak sizes at different concentration levels of the standard. The relationship can be linear, quadratic or point-to-point. Quantitation is usually based on a number of runs using a standard at several concentration levels.

The amount and concentration of the component(s) of interest in the sample are then determined from the peak size of the component using the calibration curve.

The different steps in Quantitation

For all quantitation techniques except standard addition, you need to perform the following:

1. Run the different concentration levels of the standard.
2. Integrate the curves to produce peak tables. Check the integration.
3. Identify the components for which calibration curves will be produced (see "Producing calibration curves" on page 319).
4. Enter the known concentrations for the different standards to produce a calibration curve for each selected component (see "Producing calibration curves" on page 319).
5. Run the sample and integrate the curve.
6. Let the program calculate the concentration and amount of the components of interest in the sample (see "Quantitating the sample" on page 339).

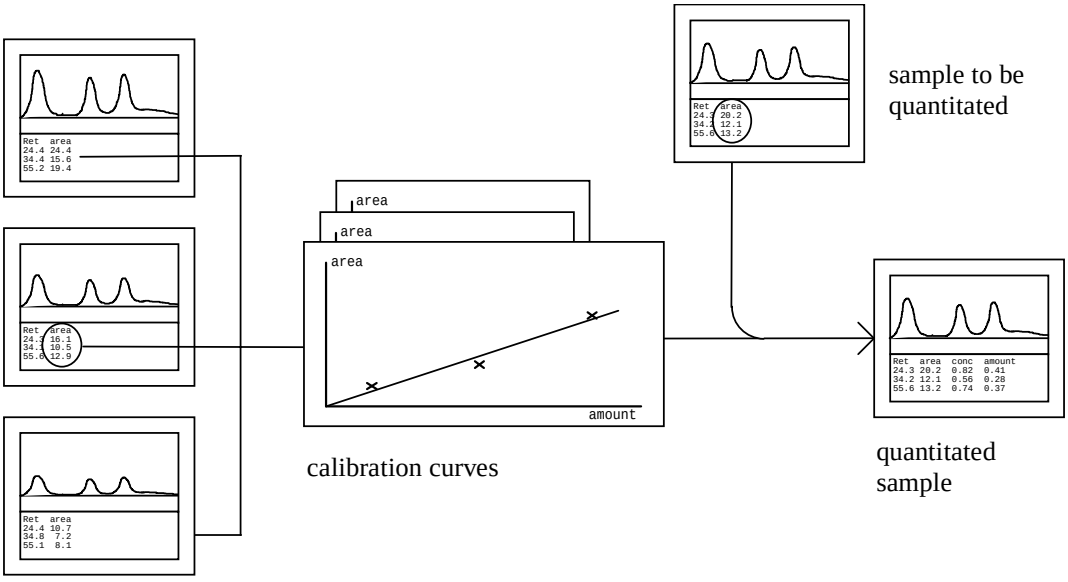


Fig 11-1. Quantitation overview.

The quantitation techniques available

The different techniques available are summarised below. Each technique is described in greater detail in the following sections.

External standard quantitation

A calibration curve is produced by running one or several concentration levels of the standard containing the component(s) of interest. The amount and concentration of the component in the sample is then determined from the calibration curve. This technique is fairly simple and usually gives good precision.

Internal standard quantitation

Peak areas of the components of interest are related to the peak area of an internal standard added in a fixed amount to each concentration level of the standard and to the sample. This technique reduces errors that are caused by changes occurring between runs and is therefore the technique that may give the highest precision if a suitable internal standard can be selected.

Quantitation by standard addition

The sample is spiked with a known amount of the component of interest. The areas of the spiked and unspiked sample are then compared and the amount in the unspiked sample is determined. No calibration curves from standards are used. Only one component can be quantitated. Compared to other techniques, standard addition enables a result to be obtained more quickly when you are performing a small number of sample runs. However, the precision is limited.

Recovery calculation

Recovery is used to determine the losses that may occur during the sample preparation process. The sample is spiked with a known amount of the component of interest. The amount in the spiked sample is then determined from a calibration curve and is compared with the amount in an unspiked sample. The recovery can only be determined for one component each time.

11.2.2 External standard quantitation

External standard quantitation is based on the use of a standard prepared in a number of concentration levels. A run is performed for each concentration level and calibration curves are produced showing the relationship between amount and peak size for each component. The calibration curves are used to quantitate the components in the sample.

The standard should contain known amounts of all the components that are to be quantitated in the sample.

The technique can be based on the use of a single standard concentration level but then the calibration curve is limited to a linear through-the-origin relationship. The use of a number of different concentration levels of the standard broadens the range of the calibration curve, allows the development of non-linear calibration curves and improves the precision. Multiple runs at each level improve precision further.

The following description is based on the use of a standard which contains two components and which is run at three different concentration levels.

An outline of the technique

1. A run is performed for each standard level.

2. The curves are peak integrated to produce a peak table for each run (for a description of the process, see "Integrating peaks" on page 259).

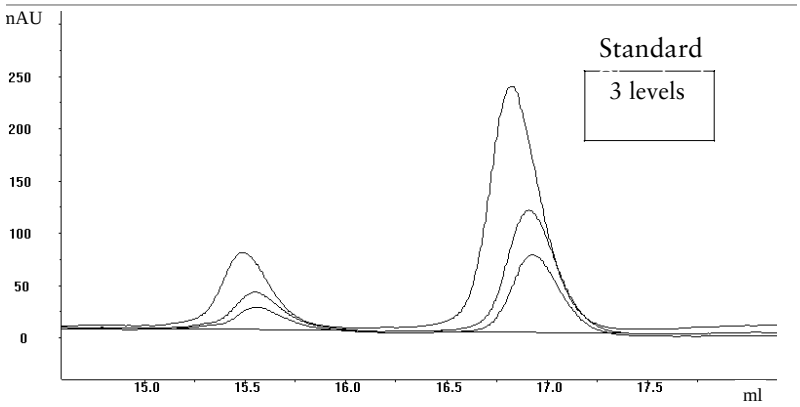


Fig 11-2. Chromatographic curves from three levels of the standard.

3. The peak tables from the series are used to produce a calibration curve for each component. This curve shows the relationship between amount and peak size. This curve is used to obtain quantity data from the sample.

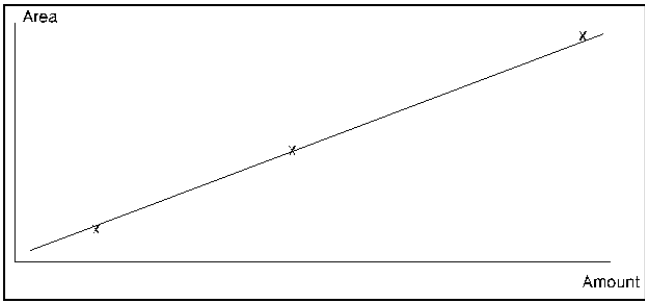


Fig 11-3. Calibration curve for one of the components, based on peak area versus amount, prepared from the standard levels.

4. A run is then performed with the sample and the curve is peak integrated.

5. The components of interest are identified from the sample peak table by the peak identification settings. Using the peak size(s), the concentration and amount are calculated from the calibration curve.

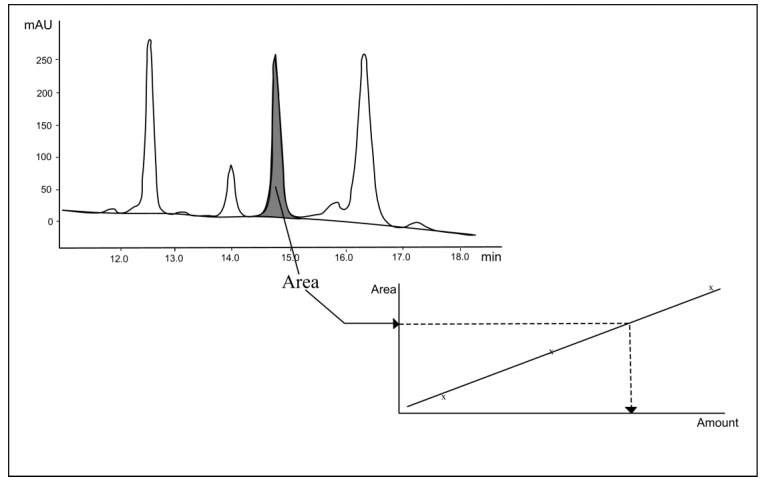


Fig 11-4. Calibration curve uses sample peak area to determine amount.

Reliability of the external standard technique

For a description of the general factors that affect the reliability, see "General factors affecting reliability" on page 318. Factors specific to the external standard technique are:

1. Precision is limited by changes that may take place between runs, e.g. column degradation and mobile phase variations.

2. There is no compensation for losses of sample during the sample preparation process prior to analysis.

However, this technique normally gives good precision and is fairly simple.

11.2.3 Internal standard quantitation

Internal standard quantitation reduces errors which are caused by changes in the system between successive runs with the sample and the standard concentration levels. For example, there may be unpredictable losses during the sample preparation procedure or unintentional changes in the amounts injected.

Internal standard quantitation uses peak tables prepared from the standard, as with external standard quantitation. However, a fixed quantity of an additional component is added to every run, including the sample. The peak sizes of the standards and the sample are then

related to the peak size of the internal standard to compensate for any changes that may have occurred between runs.

The internal standard technique relies on the assumption that any changes in the amount injected of the component(s) of interest, e.g. due to sample preparation losses, correspond to equal changes in the amount injected of the internal standard component.

A suitable internal standard:

- must be well separated from all components in the sample (not just from the components of interest)
- must not be present naturally in the sample(s).

To be able to compensate for losses during sample preparation, all the standard concentration levels must be subjected to the same sample preparation procedure as the samples. An additional demand on the internal standard is then:

- it must have similar chemical properties to the component(s) of interest. This also implies that if you have several components of interest, they all must be chemically similar.

An outline of the technique

1. A series of concentration levels is prepared from the standard. An additional component, the internal standard, is added in the same concentration to all the standards and to the sample. This addition should be made prior to the sample preparation procedure. A run is performed for each standard and the sample.

2. The curves are peak integrated to produce a peak table for all standard runs and for the sample. Each curve contains a peak from the internal standard. Changes in the size of the internal standard peak indicate changes in the system.

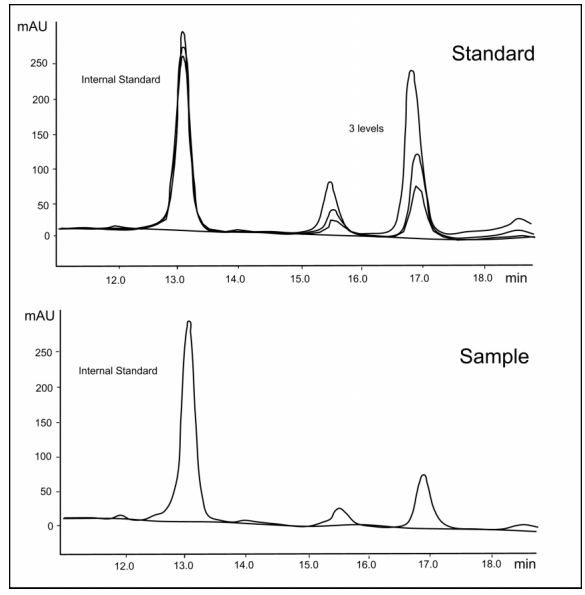


Fig 11-5. Chromatographic curves for sample and standard levels all include the peak for the internal standard.

3. All peak sizes are plotted relative to the size of the internal standard peak to produce a calibration curve for each component.

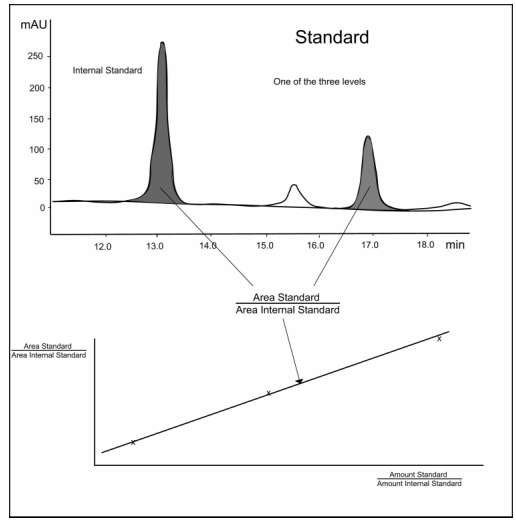


Fig 11-6. Standard peak area, relative to internal standard peak area, is used to produce a point on the calibration curve.

4. Data from the sample are prepared in the same way, producing peak sizes relative to the internal standard peak size. The resulting relative value is then applied to the calibration curve to determine the amount and concentration of the component of interest.

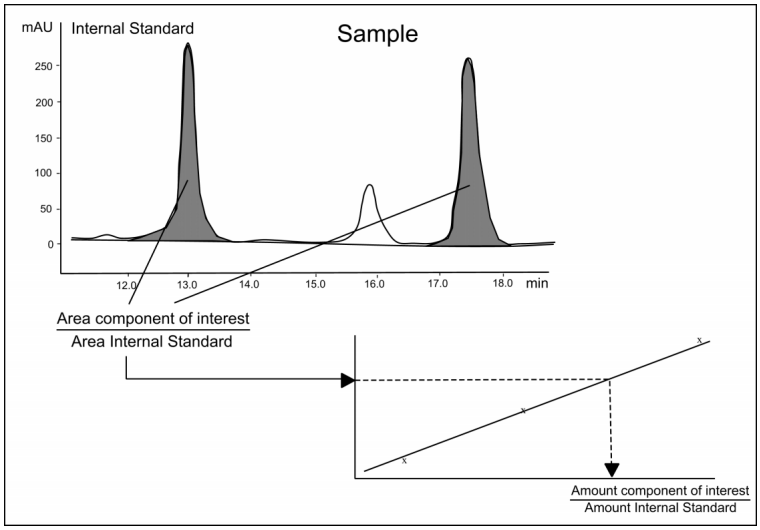


Fig 11-7. Peak area of the component of interest is related to the internal standard peak area and from the calibration curve the amount is calculated from this ratio.

Reliability of the internal standard technique

This is potentially the most reliable of the quantitation techniques. However, if the internal standard component is not carefully selected, reliability will probably be worse than for the external standard technique (see "Internal standard quantitation" on page 310 for internal standard selection guides).

Specific factors that affect reliability are:

1. If the sample contains many peaks, there is an increased risk of overlap when the extra component (internal standard) is added.

2. The addition of the internal standard must be accurate in both standards and samples, otherwise the precision of the quantitation will be reduced dramatically.

11.2.4 Standard addition quantitation

This is a simple way to obtain measurements of amount in your sample (concentration is not calculated). It requires only two runs; one with the sample and a second with the sample which has been spiked with the component of interest. The technique is straight forward and relatively quick when you are running only a few samples. Standard addition might be useful when you want to use the internal standard technique but do not have a suitable internal standard.

The disadvantages of this technique are its limited precision compared to the external and internal standard techniques and its ability to measure only one component.

An outline of the technique

1. A run is performed with the sample. The procedure for performing a run is described in Chapter 6

2. A second run is performed with a sample that has been spiked, prior to sample preparation, with a known quantity of the component of interest.

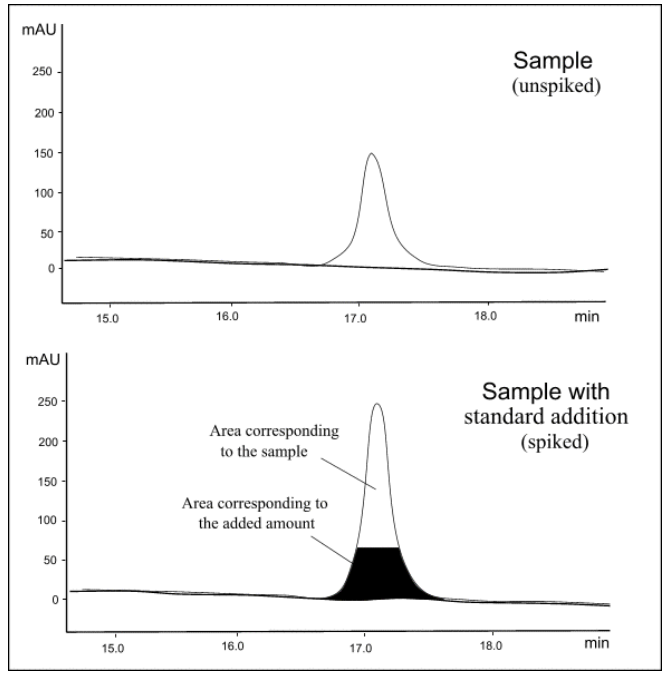


Fig 11-8. Peak areas from the sample and the sample with standard addition.

3. Using the evaluation module, a Peak integration is performed on the two curves to produce a peak table for both the spiked and unspiked sample.

4. The difference in peak area between the spiked and unspiked sample represents the peak area from the added amount.

5. Assuming linear proportionality between peak area and amount, and knowing the added amount, the software calculates the amount of the component of interest in the sample:

$$\text{Amount in unspiked sample} = \text{Amount added} \times \frac{\text{Peak area from unspiked sample}}{\text{Peak area from added amount}}$$

Reliability of the standard addition technique

Standard addition is the least precise of the quantitation techniques since it is restricted to a single concentration level and the amount in the sample is calculated by extrapolation. However, it is a useful technique when a rapid result is required and high precision is not needed and when you have only a few sample runs to perform.

- Quantitation requires that the component(s) of interest are completely resolved from all other components in the chromatogram. Overlapping peaks will give unreliable results.
- The peak integration parameters (Baseline settings) must be correctly selected. The default settings will be satisfactory in many cases, but the integration results have to be checked for all chromatograms (for additional information regarding peak integration see "Integrating peaks" on page 259). All integrations must be performed using the same X-axis base unit. Time is the recommended unit for highest reliability.
- The standard addition technique assumes a linear through-the-origin relationship between amount of component and peak size. This is a good approximation for small quantities under normal conditions.
- Changes may take place between runs. Standard addition has no way of compensating for these changes. However, if losses during sample preparation are constant between the two runs, they may be accounted for by spiking the sample prior to sample preparation.
- Precision is maximised by using a spike amount which is of the same order of magnitude as the sample.
- Precision is maximised by performing all runs consecutively and thereby reducing systematic errors.

11.2.5 Recovery calculation

Recovery is used to determine losses which may occur during the sample preparation process. Recovery can also be used to determine the recovery factor of a preparative purification or chromatographic process. The recovery can only be determined for a single component.

A calibration curve is produced using a concentration series of an external standard. The calibration curve range must cover the amount in both the sample and the spiked sample. Two runs are performed, one with the sample and the second with the sample that was spiked with a known amount of the component of interest prior to sample preparation. Quantitation of the data from the two sample runs allows the recovery factor of the sample preparation to be calculated.

The recovery is measured as the recovery for the sample preparation, not for the separation during the chromatographic analysis.

An outline of the technique

1. A run is performed with each level of the standard.

2. The curves are peak integrated to produce a peak table for each level.

3. The data from the peak tables are used to produce a calibration curve. This is the same process as is used in external standard quantitation.

4. A portion of the sample is spiked with a known amount of the component of interest prior to sample preparation. Both an unspiked and a spiked sample are then run and peak integrated to produce, respectively, the sample and sample-with-addition peak tables.

5. The amounts for unspiked and spiked sample are calculated from the calibration curve. The difference between these amounts provides the apparent amount of the addition.

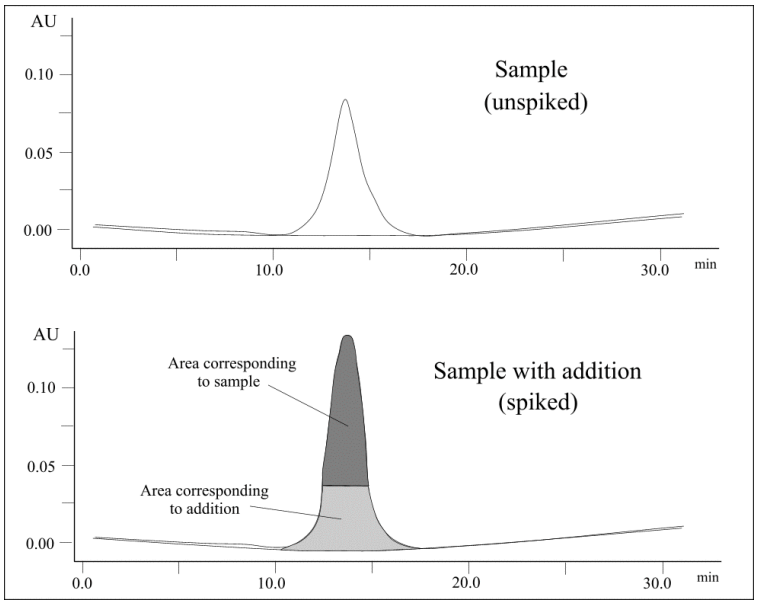


Fig 11-9. The apparent amount of the addition is calculated by subtracting the amount of the unspiked sample from the amount of the spiked sample.

6. The ratio of this apparent amount to the amount actually added to the sample determines the recovery of the system.

$$\text{Recovery factor} = \frac{\text{Apparent amount added}}{\text{Actual amount added}} \ ^{*}$$

* Apparent amount added = Amount of spiked sample - Amount of unspiked sample

For example, if 2 mg of the component of interest had been added to the sample and quantitation indicated an apparent amount added of 1.6 mg, the recovery factor was 0.8.

The recovery factor may be used to manually compensate for losses during sample preparation. The apparent amount in a sample is divided by the recovery factor to obtain the corrected amount.

Reliability of the recovery factor result

Specific factors that affect reliability are:

1. For good precision, the sample should be spiked with an amount of the component of interest in the same order of magnitude as the sample.

2. It is assumed that the recovery is the same both for the sample and the spiked sample. However if the recovery varies according to the amount of the component of interest, results are unreliable.

11.2.6 General factors affecting reliability

These factors are valid for all techniques apart from standard addition.

- Quantitation requires that the component(s) of interest are completely resolved from all other components in the chromatogram. Overlapping peaks will give unreliable results.
- The peak integration parameters (Baseline settings) must be correctly selected. The default settings will be satisfactory in many cases, but the integration results have to be checked for all chromatograms (for additional information regarding peak integration, see "Integrating peaks" on page 259). All integrations must be performed using the same X-axis base unit. Time is the recommended unit for highest reliability.
- The concentration levels of the standard have to be accurately prepared. Errors in the amount or concentration values will lead to unpredictable results.
- Self imposed limitations, such as the use of a small number of concentration levels of the standard, also limit precision.
- Appropriate choice of the concentration range of the standard will improve precision. This should extend across the presumed amount in the sample.
- Use of the most appropriate curve model will maximise precision.
- The accuracy is improved if several runs are performed at each level.
- Precision is maximised by performing all runs consecutively and thereby reducing systematic errors.

For detailed information about quantitative analysis, see a statistical textbook such as "Statistics for Analytical Chemistry", 3rd Edition 1993, J.C. Miller and J.N. Miller, Ellis Horwood PTR Prentice Hall.

11.3 Producing calibration curves

This section describes how peak data from standards are used to prepare calibration curves for use with external standard, internal standard and recovery quantitation.

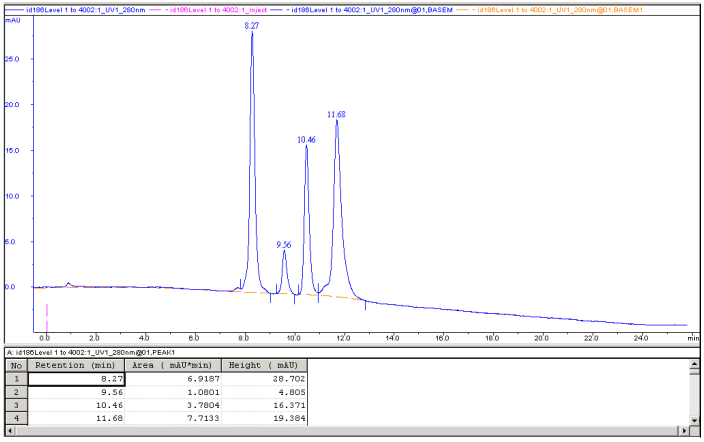
11.3.1 Preparations before using Quantitate

1. Create a method to be used for all the standard runs. The method and the injection volume must be the same for all the runs.

   If the method is created from a Wizard or template for ÄKTAdesign systems, you may select the correct standard concentration level in the variable **Quantitation_Type**. You can also set the level after the run has been performed (see "Editing and updating a quantitation table" on page 334). Each level is an alias for a specific concentration of the standard. Level 1 should be selected for the standard with the highest concentration. The levels must be set in consecutive order of decreasing concentration of the standard. All runs with the same concentration must be given the same level.

2. Perform at least one run for each concentration level of the standard. Preferably the standard series should include standard concentrations which extend beyond the lower and upper limits of the sample amount. If internal standard is being used, the internal standard must be added in the same concentration in all standards.

3. Using the Evaluation module, peak integrate the curves to produce a peak table for each of the standard curves. When integrated, all standards must use the same X-axis base unit. Time is the recommended unit for highest reliability.



No	Retention (min)	Area (mAU*min)	Height (mAU)
1	8.27	6.9187	28.702
2	9.56	1.0801	4.805
3	10.46	3.7804	16.371
4	11.68	7.7133	19.384

Fig 11-10. Curves window showing peak data from a standard curve.

4. Check that each integration is correct and consistent. If not, see "Optimising peak integration" on page 262 for directions on how to optimise the integrations.

   If many small irrelevant peaks are detected, it may be an advantage to re-integrate after adjusting the Reject peaks criteria. The number of largest peaks to detect has a default of 20 and it may be helpful if this is set to a smaller value.

5. Use **File:Save** to save all the peak tables.

That completes preparations prior to the use of Quantitate.

11.3.2 Creating a quantitation table

The quantitation table contains all the necessary data, such as the calibration curves, needed to quantitate one or several components in a sample.

The procedure for creating quantitation tables is the same for both external standard quantitation and for recovery calculations. They both use absolute values of standard peak data. For quantitation with internal standard, the peak sizes relative to the size of the internal standard peak are used to create a calibration curve. Please see "Internal standard quantitation" on page 310 for more information.

Opening the result file

1. From the **Main Menu**, highlight the result file which contains the peak table data from the standard level with the highest concentration.

2. Select **File:Open** or double click on the result file name.

3. The **Evaluation** window opens together with the curves window showing the curves and peak tables.

4. Select **Properties** for the chromatogram window and use the **Chromatogram Layout** dialogue to select the curves and peak table parameters for display.

Entering the standard data

1. From the **Evaluation** window menu bar select **Quantitate:Edit Quantitation Table:New**.

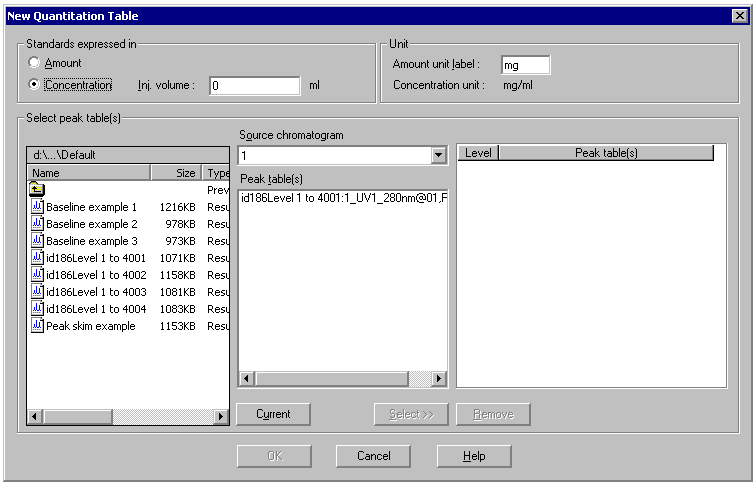


Fig 11-11. New Quantitation Table dialogue.

2. If the standards are to be expressed in concentration rather than amount, click **Concentration** and check or edit the injection volume in the **Inj. Volume** field. Note that the software will always calculate both amount and concentration for the sample.

3. The default **Amount unit label** is mg. Edit this if necessary.

4. The name of the active chromatogram should appear in the **Source chromatogram** field. If you need to use an alternative result file, double click on its icon within the **Result** field and select the source chromatogram. Clicking **Current** at any time returns you to the chromatogram that was active before entering Quantitate.

5. Highlight the standard peak table of level 1 in the **Peak table(s)** list. This should be the table for the highest concentration of the standard.

6. Click **Select** (or double click the peak table) to include it in the **Level/Peak table(s)** list. If the level has already been set in the method, the level is automatically copied into the **Level/Peak table(s)** list. If not, a **Select Level** dialogue will appear. Open the **Level** menu, highlight 1 and click **OK**. It is useful to think of each level as an alias for a specific concentration of the standard.

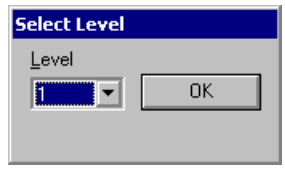


Fig 11-12. Select Level dialogue.

7. Repeat this procedure with all the standard peak tables. Open another result file by clicking on its icon in the **Results** field and then select the new source chromatogram. The peak tables associated with this chromatogram appear in the **Peak table(s)** list.

   Increment the level number for each new standard concentration (if not set automatically). The levels must be in consecutive order of decreasing concentration of the standard. You can incorporate up to 10 peak tables at each level, prepared from runs repeated at the same concentration. Quantitate will later allocate each with an incrementing suffix, e.g. 1:1, 1:2 etc.

8. If you wish to remove a selected table from the list, highlight it and click **Remove**.

9. Click **OK** and the **New Quantitation Table** dialogue is replaced by the **Define component(s)** dialogue.

Examining the components

You use the **Define Component(s)** dialogue to select the components that will be used to produce the calibration curve(s). Quantitate must be able to identify these components in all levels. Setting the criteria by which peaks are identified is a part of this dialogue.

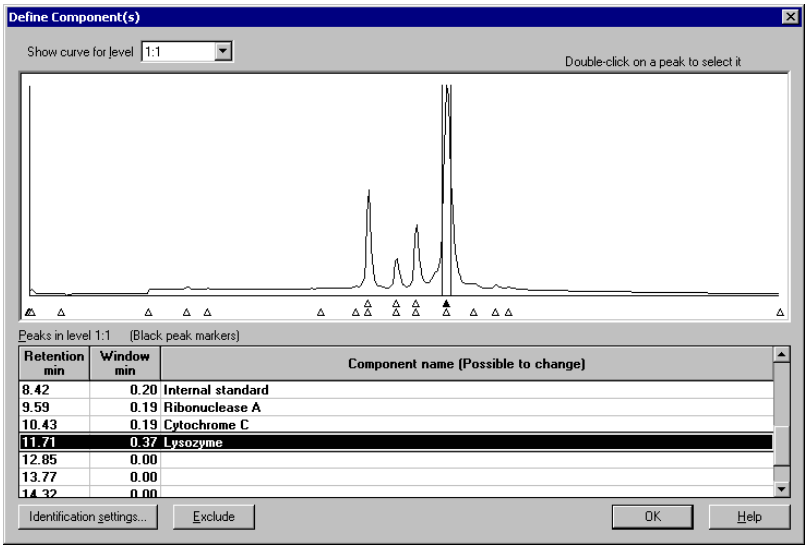


Fig 11-13. Define Component(s) dialogue.

Looking at the contents of each concentration level

Initially the **Define Component(s)** window displays the components from level 1:1, the peak table from the highest concentration of the standard. Use the **Show curve for level** menu to examine the curve for each standard run. As you select levels down the list there is a progressive reduction in the size of the components, reflecting the decreasing concentration of the standard.

If an internal standard has been incorporated, its peak remains about the same size in each level.

Selecting the components

Use level 1:1 to select the components. Each peak detected during the peak integration, i.e. present in the peak table, is identified by a lower triangle (black in level 1:1, green for other levels). There may be different peaks detected for different levels. Upper triangles will later identify peaks selected for quantitation.

1. Click on a peak to highlight its position in the table.

2. Double-click on a peak or click on **Include** to select it for quantitation. More than one peak can be selected to produce calibration curves for several components of interest.

3. Selected peaks will be shown with an upper triangle and with the name “component no.” in the table. The selected peaks are the same in all levels.

4. Change the component name by marking it and enter a new one.

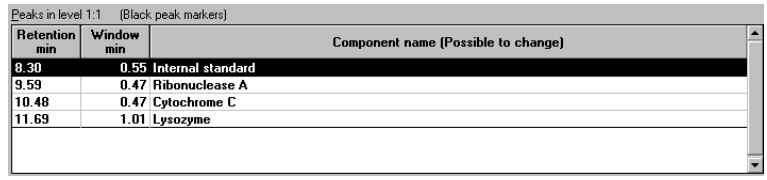
5. When clicking on a selected peak, vertical cursor lines will show its identification window (see below).

6. If you have an internal standard, select it by double-clicking on it and give it a new name.

7. If you want to exclude a selected peak, mark it and press **Exclude** (or double click on the peak again). Excluding a peak in one level excludes it from all levels.

The Peak table within the **Define component(s)** dialogue shows three columns:

- the (absolute) **Retention** value of the component in level 1:1.
- the width of each components window. If you change the width of a window by adjusting the cursor lines, it will be reflected in the **Window** column.
- the list of components with the currently selected component highlighted.


Peaks in level 1:1 (Black peak markers)

Retention min	Window min	Component name (Possible to change)
8.30	0.55	Internal standard
9.59	0.47	Ribonuclease A
10.48	0.47	Cytochrome C
11.69	1.01	Lysozyme

Fig 11-14. Peak table columns.

Peak identification

When a component is selected, vertical cursor lines show the current identification window. The software uses this window to search for peaks in other levels and in the sample run(s). A peak found in the window is assumed to be the component of interest. You can change the limits by dragging a limit cursor line. Both cursor lines move symmetrically so that the limits centre on the component peak.

1. Set the window to a suitable width. The window should be set wide enough to include peaks in the other levels, despite minor variations in retention volumes. However, the window should also

be narrow enough to exclude unwanted peaks which will interfere with the quantitation.

2. Go through the other levels and check that the width is suitable (the window width is the same in all levels). To display the actual retention for a peak, click on its lower green or black triangle.

3. Repeat for all selected peaks. Overlapping windows are not allowed.

By default, peaks are identified by their absolute retention values and the highest peak maximum within the window. In most cases it is not necessary to change these default settings in which case you can click on **OK** and move directly to the IS and settings section on page 328.

If you have drifting retention and/or the presence of nearby or slightly overlapping peaks which may cause the wrong peak to be selected, you may need to change the identification settings.

The criteria by which peaks are identified are set using the **Identification Settings** dialogue. The criteria are valid for all the selected peaks in the **Define Component(s)** dialogue. These settings also affect the information provided in the **Peak table** within the **Define Component(s)** dialogue.

Click on **Identification Settings** to open the dialogue.

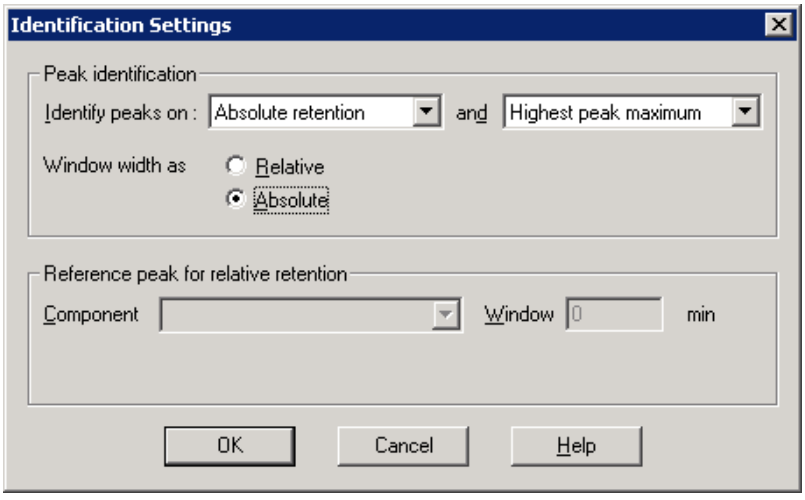


Fig 11-15. Identification settings dialogue.

Peak identification by absolute retention

Use this option when there has been little or no drift in retention between successive runs of the standard. Quantitate will find corresponding peaks in these successive runs providing any drift in retention does not move a peak outside the peak window.

Peak identification by relative retention

If peak identification becomes difficult because of drifting retention between runs which moves peaks out of the peak window, you can choose to identify peaks according to their position relative to a reference peak. This is achieved as follows:

1. Open the **Define Component(s)** dialogue again and identify a component for use as the reference. Choose a peak that is well separated from any other peaks. This enables the window to be set relatively wide and the system can accommodate a larger drift in retention value.

2. Click **Identification settings** and set **Identify peak on** to **Relative retention**.

3. Open the **Component** menu and select the component to be used as the reference peak and set the window width for that peak (an absolute value). The width should be set fairly wide to be able to accommodate a larger drift. Make sure there are no other large peaks within the window.

4. Click **OK** to return to the **Define Component(s)** dialogue.

The **Peak table** now includes another column, **Ret/Ref**, which shows the retention value of each component relative to the retention value of the reference component. This reference component is marked "Ref." in the **Window %** column. The **Window %** column shows the window width for each peak expressed as a percentage of its relative retention value.

Peaks in level 1:1 (Black peak markers)

Retention min	Ret / Ref	Window %	Component name (Possible to change)
8.42	0.72	2.38	Internal standard
9.59	0.82	1.98	Ribonuclease A
10.43	0.89	1.82	Cytochrome C
11.71	1.00	Ref	Lysozyme

Fig 11-16. Peak table columns for relative retention.

Identifying a peak within a window

If any of the windows shown in the **Define Component(s)** dialogue includes more than one peak, Quantitate must be advised how peaks are to be identified. Use the second Peak identification menu in the **Identification settings** dialogue which offers the following options:

- Highest peak maximum (the default)
- Closest to retention, i.e. closest to the centre of the window (see retention column in the table in the **Define component(s)** dialogue). Avoid this option in combination with relative retention
- Maximum peak area.

Examine the nature of the peaks enclosed by the window then select the option which differentiates between the wanted and unwanted peaks. If there are large peaks from components which are not going to be quantitated, use **closest to retention**.

The selection applies to all peaks, even the internal standard and reference peaks if used.

Absolute and Relative window width

While **Peak identification** is set to **Absolute retention**, the peak window width can be displayed as **Absolute** or **Relative** by clicking the appropriate button in the **Identification Settings** dialogue.

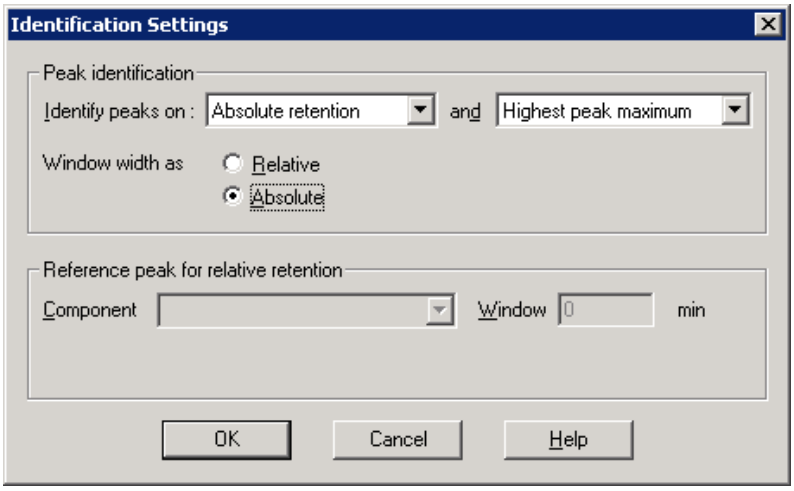


Fig 11-17. Identification settings dialogue.

Select **Absolute** to show the window width of each peak in minutes (or ml). Select **Relative** to display the width of each component as a percentage of its retention.

If **Peak identification** is set to **Relative retention**, **Window** is set automatically to **Relative** except for the reference peak.

When component selection and identification setting is complete, click **OK** to open the **Quantitation table** dialogue.

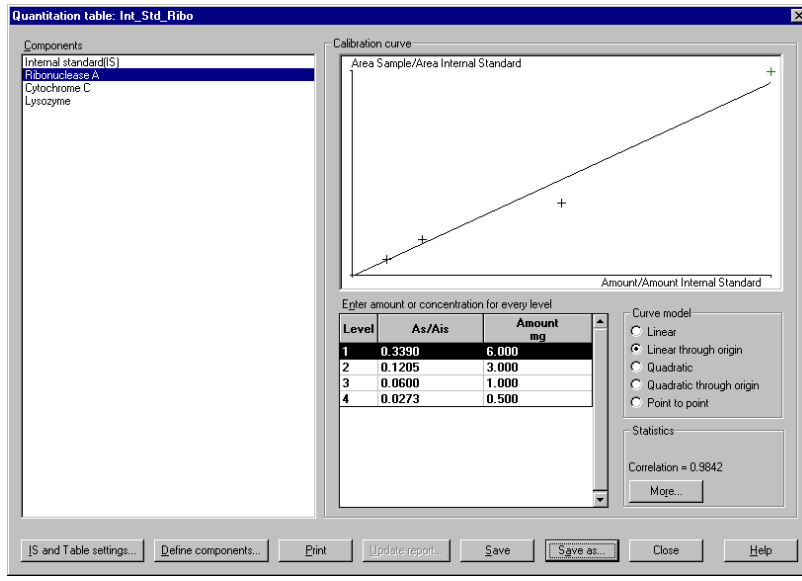


Fig 11-18. Quantitation table dialogue.

Calibration curves will now be produced. If internal standard quantitation is used, the internal standard must first be selected within the **Table settings** dialogue. This dialogue also allows you to set the basis of the calibration curve on peak area (the default) or peak height. If internal standard is not used, you normally do not need to make any changes in the **Table settings** dialogue in which case you can move on to "Entering the data for the standards" on page 329.

IS and settings

To select the internal standard, click **IS and Table settings** in the **Quantitation table** dialogue.

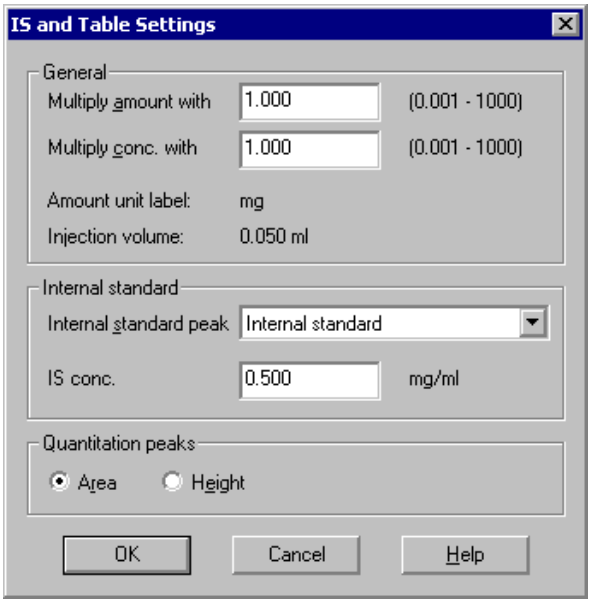


Fig 11-19. IS and Table settings dialogue.

Use this dialogue to enter:

- The amount and concentration multipliers you wish to apply. When the calibration curve is applied to a sample, the amount and concentration of the sample are multiplied by the values. The multipliers should normally be set to 1. Change them if you want to determine the amount or concentration in the starting volume of the sample instead of in the injected volume of the sample.
- Information about any internal standard being used. If you are preparing for external standard quantitation or measurement of recovery factor, the default option **Not selected** should be chosen. If internal standard quantitation is being used, open the **Internal standard peak** menu and select the component which represents the Internal standard. Next enter the injected **IS amount** (or **IS concentration**) in the standard and sample runs.
- Quantitate can base the calibration curve on peak area or peak height. Peak area is usually used and this is the default. You may wish to change **Quantitation peaks** to **Height** if peaks are not completely separated from those of other components.

Entering the data for the standards

Check that the components selected are correct. If you wish to edit the list, click **Define components** to return to the **Define Component(s)** dialogue.

If relative retention has been used, the reference component is marked **(Ref)**.

If an internal standard is in use, the related component is labelled (**IS**).

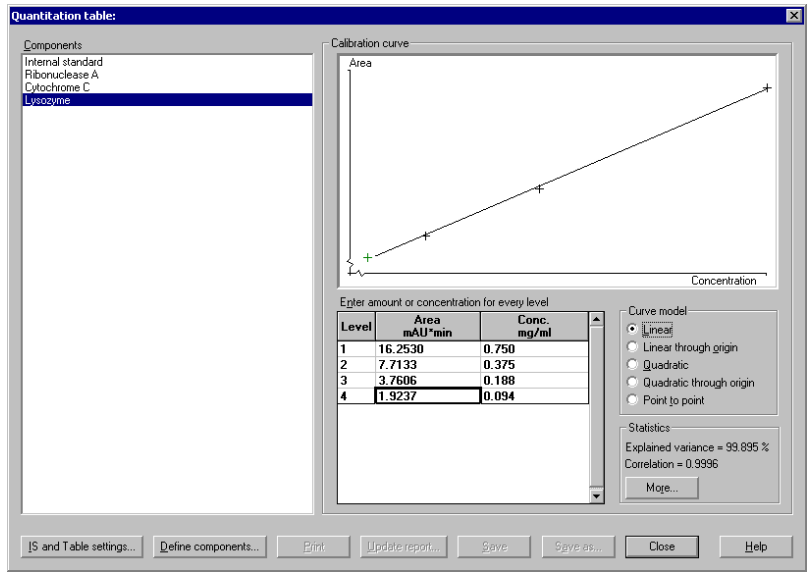


Fig 11-20. Quantitation table dialogue.

Select each component in turn and enter the amounts at each level:

1. From within the **Quantitation table** dialogue, select the first component at the top of the **Components** menu. Do not select an internal standard component (if available) as the amount for this has already been entered and does not change between the levels.

2. Double click in the first row, Level 1, in the **Amount** column and insert the amount of the component in the standard at this level.

Note: This is the amount corresponding to the injected volume, not the total amount used when the standard level was prepared.

3. Repeat this operation for this component at the other levels. You can use the cursor keys to move rapidly between levels in this column.

4. Use the **Curve model** radio buttons to select the curve model that gives the best fit:

- Linear (recommended)
- Linear through origin
- Quadratic
- Quadratic through origin
- Point to point

The resulting curve is shown in the **Calibration curve** window together with points representing each of the levels of the component. Any of the points in this window can be selected by highlighting its entry in the table underneath.

If more than one run has been performed for some level, all points in that level will be shown. However, the average of these points will be calculated and then this average value will be used for producing the calibration curve. For more information on the curve fit, click on **Statistics** to open the **Statistics** information box. For mathematical and statistical details, see "Mathematical models and statistics" on page 405.

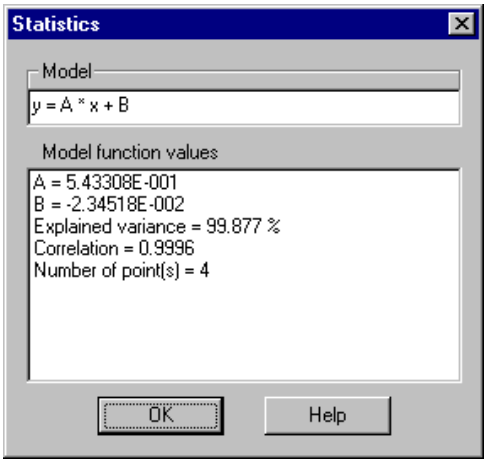


Fig 11-21. Statistics dialogue.

The correlation, which is only displayed for linear models, should be as close as possible to 1.00.

The best model may be selected by testing different models and comparing the explained variance values but you still need to examine the calibration curve visually to ensure a good fit. The explained variance value should be as close as possible to 100% but is usually rather high even for poor models. For instance, a value of 90%

indicates a very poor model. The explained variance will not be shown for curve models drawn through the origin.

If the point-to-point model is selected, no statistics are available.

5. Complete the quantitation table by entering data and the curve model for the remaining components in the **Components** list. You will then have one calibration curve for each component.

   The data must be entered and models selected for all components before the Quantitation table can be saved.

Note: If relative retention is used for peak identification and the reference peak is not used for quantitation, amounts must still be entered for the reference peak and a model must be selected before the quantitation table can be saved. Enter dummy values and select any model.

Saving the quantitation table

As this is a new table, it can only be saved at this stage by use of the **Save as** button.

Fig 11-22. Save quantitation table dialogue.

The **Save quantitation table** dialogue provides a listing of existing quantitation tables and enables you to enter the name for the new quantitation table.

1. You can choose whether the table will be globally accessible to any user or only to someone using your user id. The default is **Global**. Click **Personal** if access is to be restricted.
2. Enter a name for the table. This name may be up to 20 characters long and may contain letters A-Z, digits 0-9 and the underscore character.
3. Click **OK** to save the table.

Note: Once the table is saved under **Save as**, it can be updated using the same name by use of the **Save** button. Care is needed as this overwrites the original table. You might prefer to use **Save as** and a new name after any editing operations and so preserve the original table.

4. Click **Exit** to leave Quantitate.

Printing the quantitation table.

The **Quantitation table** dialogue provides a print function which you can use to print the data on all components and their related calibration curves.

Click on **Print** to print the calibration curves.

To print a quantitation table that was prepared previously:

1. From the **Evaluation** window, select **Quantitate:Edit Quantitation Table:Open.**

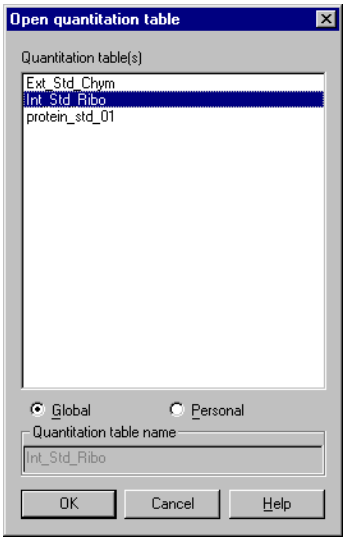


Fig 11-23. The Open quantitation table dialogue.

2. Select **Personal** if necessary then highlight the quantitation table required and click **OK**.

3. The **Quantitation table** dialogue opens. The **Components** field lists all the components for which quantitation data are available. Click **Print** to print the data.

Deleting a quantitation table

1. From **Evaluation**, select **Quantitate:Edit Quantitation Table:Delete** to open the **Delete Quantitation table(s)** dialogue.

2. Select **Global** or **Personal** as required to access the table you wish to delete.

3. Highlight the table in the list.

4. Click **Delete** to delete the table, or **Exit** to abort the delete.

Renaming a quantitation table

1. From **Evaluation**, select **Quantitate:Edit Quantitation Table:Rename** to open the **Rename Quantitation table(s)** dialogue.

2. Select **Global** or **Personal** as required to access the table you wish to rename.

3. Select the table you wish to rename.

4. Click in the **Quantitation table name** field and type the new name.

5. Click **Rename** to rename the table, or **Exit** to abort the rename procedure.

11.3.3 Editing and updating a quantitation table

Quantitate has an editing facility that allows you to return to a quantitation table and alter any of the parameters. Note the difference between this function and **Update** which allows you to incorporate new data into a pre-existing quantitation table. **Update** is described in below.

To edit a quantitation table:

1. Select **Quantitate:Edit Quantitation Table:Open** from the Evaluation window.

2. Use the **Open quantitation table** dialogue to select the quantitation table you wish to edit.

Selection of the table opens the **Quantitation table** dialogue. The editing facility gives you full access to all the quantitation table parameters.

If you have used the **Update** function (see below), it is not possible to make changes in the **Define Component(s)** dialogue.

3. Once editing is complete, click **Save** or **Save as**, depending on whether you wish to overwrite the existing table or create a new table under a new name.

Updating a quantitation table

Quantitate includes an update function which allows you to add new peak size data to an existing quantitation table. This enables precision to be improved through the use of data from a number of standard runs and also simplifies the process of renewing the calibration curves before each analysis.

The injection volume must always be the same for the new run as it was for the previous standard runs.

Proceed as follows to initiate the update procedure:

1. Select **Quantitate:Edit Quantitation Table:Update** from the Evaluation window. This opens the **Update Quantitation Table** dialogue.

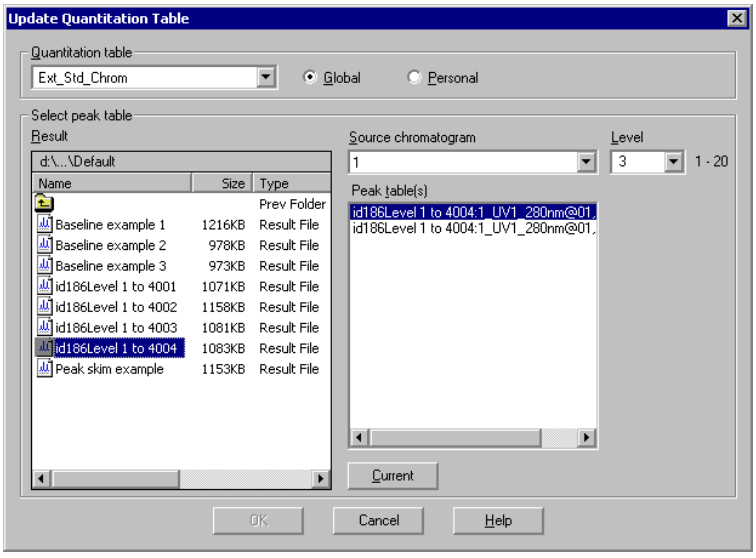


Fig 11-24. Update Quantitation Table dialogue.

2. Select **Personal** if the quantitation table is located in your personal folder.

3. Open the **Quantitation table** menu and highlight the table which is to be updated.

4. Double click the result file icon to access the new data, or click **Current** if you wish to use the result file already open in **Evaluation**.

5. Use the **Source chromatogram** menu to select the source chromatogram required and highlight the peak table which contains the new data.

6. If the level has not already been set in the method, open the **Level** menu and select the level which you wish to update.

7. If the selected quantitation table is based on concentration, check or edit the **Inj. Volume** field

8. Click **OK** and the **Update calibration curve** dialogue opens.

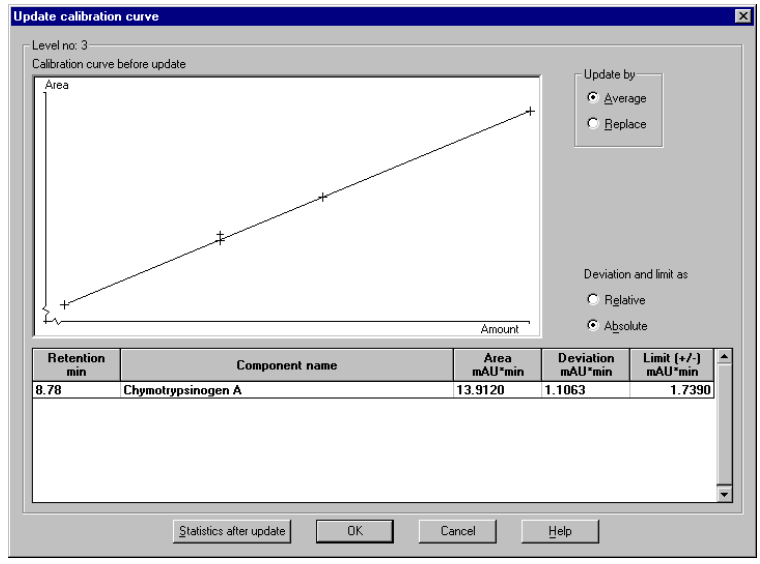


Fig 11-25. Update calibration curve dialogue.

Data on the selected components for the curve to be updated are shown in the table. When a component is highlighted in this table, its calibration curve is displayed in the window above.

The calibration curve to be updated is shown without taking the new point into consideration. A new point is shown either in green or red. If it is green, the area falls within the set **Limit (+/-)** value and this point will be used for calculation of the new calibration curve, instead of the old point. If it is red, it falls outside this range and will not be used for update.

The **Deviation** column shows how much the peak size for the proposed new point differs from the existing size. The **Limit (+/-)** shows the set limit for the deviation. The default value is +/-12.5% of the existing peak size. You can edit the **Limit (+/-)** value. Both of these columns can be expressed in **Absolute** or **Relative** (%) units by use of the **Deviation and limit as** buttons.

The update can be made by **Average** or **Replace**.

With **Average**, the average area value is calculated from the old point (representing the average of the old points at this level) together with the new point. The green point represents this new average value and not the position of the point from the new peak table. Update by **Average** may be used if you want to increase the precision of the calibration curve by performing several runs at each level.

With **Replace**, the old point (representing the average of the old points at this level) will be replaced with the new point shown in green. The data for the old point can then not be recovered. Update by **Replace** may be used to simplify the process of renewing the calibration curve before each analysis. Instead of manually producing a new quantitation table, you may renew an existing table by running all standard levels again and updating the table with Replace. The old data will then be deleted.

You use this dialogue as follows:

1. Select to update by **Average** or **Replace**. The same selection applies to all components.

2. Highlight each component in turn and check that the new point falls within acceptable limits.

3. Click **Statistics after update** to open the **Statistics after update** dialogue.

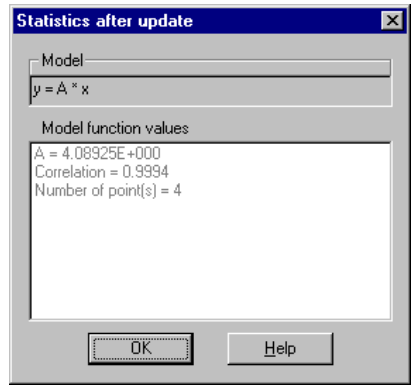


Fig 11-26. Statistics after update dialogue.

4. Use the statistical data to check the curve model after the update. Note that the old non-updated calibration curve is still shown but the statistics apply to the data after the update. If the new point is red, the statistics shown will be those for the old curve.

5. Click **OK** to close this dialogue.

6. Repeat this procedure for each component.

7. Click **OK** in the **Update calibration curve** dialogue to progress the update and open the **Update report** dialogue.

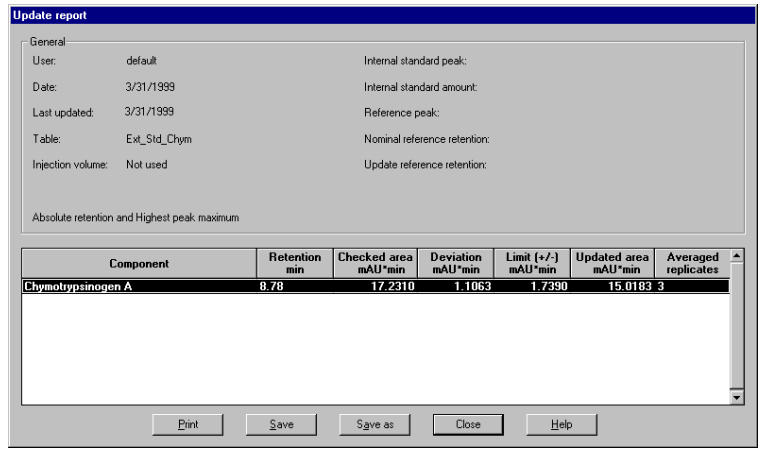


Fig 11-27. Update report dialogue.

This report provides a summary of the proposed update so that you can assess its viability.

Components that will not be updated are shown in the column **Updated area** (or **Updated ratio** if internal standard is used) with the text "Out of limit". If the text "Not found" appears, no peak was found within the set peak window.

The column **Averaged replicates** shows the number of points used to calculate the average area value. After each update by **Average**, the number is increased by one. After an update by **Replace**, the number will be one.

Nominal reference retention shows the retention for the reference peak in level 1:1.

Update reference retention shows the retention for the reference peak in the new peak table.

- Use **Save** or **Save as**, depending on whether you wish to overwrite the existing table or create a new table under a new name.
- Click **Print** to obtain a print-out of the **Update report**.

Click **Exit** if you do not wish to proceed with the update or to close the dialogue after saving the table.

11.4 Quantitating the sample

This section describes how samples are quantitated using calibration curves.

Calibration curves are applicable to external and internal standard quantitation and to recovery factor measurement.

Standard addition measurements are also described.

Note that the method used for the sample runs must be the same as for the standard runs. If the method was created from a Wizard or template for ÄKTAdesign systems, select **Sample** in the variable **Quantitation_Type**.

11.4.1 Quantitation by external and internal standard

The processes involved in external or internal standard quantitation of a sample are very similar. The procedural differences occur mainly during the creation of the quantitation tables.

A quantitation table is specific to either internal or external standard quantitation.

For an explanation of the external and internal standard techniques, please see "External standard quantitation" on page 308 and "Internal standard quantitation" on page 310, respectively.

Preparing for quantitation

A quantitation table for the components of interest must first have been prepared ("Creating a quantitation table" on page 320).

1. Perform a run with the sample. If internal standard quantitation is being used, the internal standard must have been added to the sample prior to the sample preparation procedure. The injected amount must be the same as in the standard levels.
2. Open the sample result file and peak integrate the sample curve to produce a peak table. During integration, the sample curve must use the same X-axis base unit as the standards. Time is the recommended unit for highest reliability.
3. Use **File:Save** to save the peak table before continuing with the quantitation.

If you want to show the names of the components above the peaks, open the **Chromatogram layout** box, click on **Peak Label**, select **Peak name** and click **OK**.

Calculating the amount and concentration in the sample

1. From Evaluation, select **Quantitate:Calculate amount and conc**. This opens the **Calculate Amount and Concentration** dialogue.

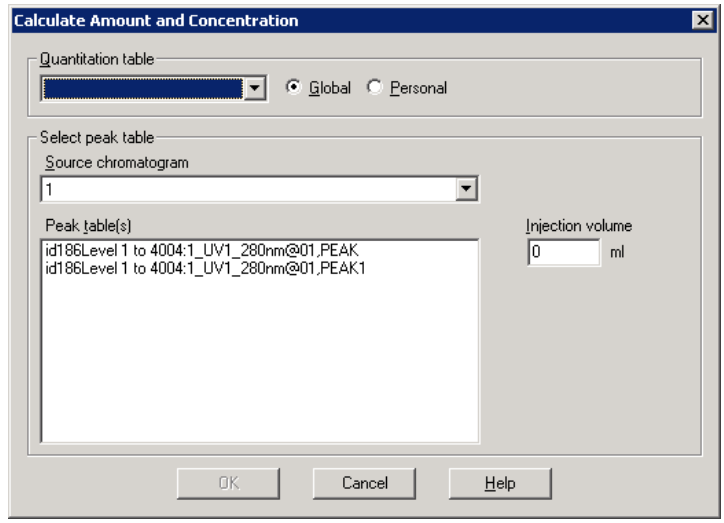


Fig 11-28. Quantitate Amount and Concentration dialogue.

2. From the **Quantitation table** drop-down list box, select the quantitation table to be used. If the table was saved using the personal option, click the **Personal** button before opening the drop-down list.

3. Use the **Source chromatogram** drop-down list box to select the source chromatogram which contains the sample curve.

4. Select the sample peak table from the **Peak table(s)** list.

5. Check the **Injection volume**. Enter or edit this if necessary. For internal standard quantitation, the injection volume must be the same as used for the standard runs.

6. Click **OK** to complete the **Quantitate** dialogue.

Viewing the results of quantitation

The results of quantitation are shown in the **Amount** and **Concentration** peak table columns.

No	Peak name	Retention (min)	Area (mAU*min)	Height (mAU)	Conc (mg/ml)	Amount (mg)
11	Ribonuclease A	9.59	12.9160	49.212	0.405	0.203
12	Cytochrome C	10.43	28.1310	101.444	0.416	0.208
13	Lysozyme	11.71	139.5769	324.616	0.612	0.306

Fig 11-29. Part of the peak table showing amount and concentration values for quantitated peaks.

If the amount can not be calculated, one of the following signs is shown in the **Amount** column:

> means that the value is higher than the highest value in the calibration curve, i.e. it is outside the valid range of the calibration curve

< means that the value is lower than the lowest value in the calibration curve, i.e. it is outside the valid range of the calibration curve

\- means that the value can not be calculated. For example, this sign might indicate that the peak could not be identified.

When the result file is saved, it includes the quantitation table that was used for the quantitation. You can later show the table that was used by selecting **Quantitate:Edit Quantitation Table:View Current**.

If you want to print the table that was used, select **File:Report** and select the **Quantitate and Mol. Size** option (see "Printing reports" on page 232).

11.4.2 Quantitating by standard addition

For an explanation of the standard addition technique, see "Standard addition quantitation" on page 314. There are four stages to the process:

1. performing the two runs and then copying the curves into one result file
2. integrating the curves to produce the peak tables
3. selecting the component to be used
4. evaluating the amount of a component in the sample.

Preparing for quantitation

1. Perform a run with both the spiked and unspiked sample.
2. The result file you use must contain the curves for both the spiked and unspiked samples. Open one of the two result files. Use **File:Open:Curves** to copy the second curve to the opened result file.
3. Peak integrate the curves to produce the peak tables for the unspiked and the spiked sample. Please refer to Chapter 10 for details on peak integration. During integration, the sample curves must use the same X-axis base unit. Time is the recommended unit for highest reliability.
4. Check that the integrations are correct. If not, consult "Optimising peak integration" on page 262 which describes how to optimise integrations.
5. Use **File:Save** to save the peak tables before continuing with the quantitation.

Selecting the component to be used

1. Select **Quantitate:Standard Addition**. This opens the **Standard Addition** dialogue.

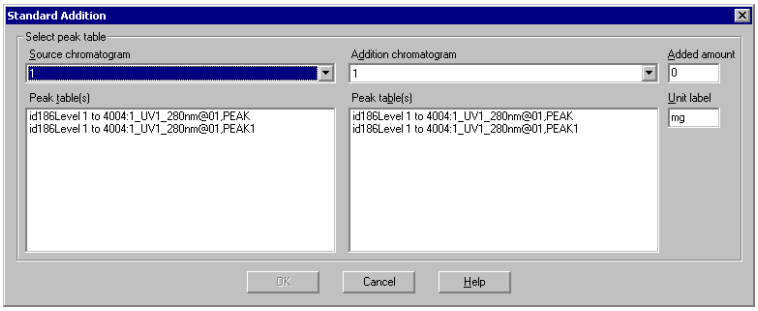


Fig 11-30. Standard Addition dialogue.

2. Open the **Source chromatogram** drop-down list and select the chromatogram which contains the peak table for the unspiked sample.

3. Highlight, in the **Peak table(s)** list, the peak table for the sample.

4. Follow the same procedure in the **Addition chromatogram** section to select the addition peak table for the spiked sample.

5. Enter in the **Added amount** field the amount of the component which was added as the spike.

6. Edit, if necessary, the default unit 'mg' in the **Unit label** field.

7. Click **OK** to open the **Identify Peak** dialogue.

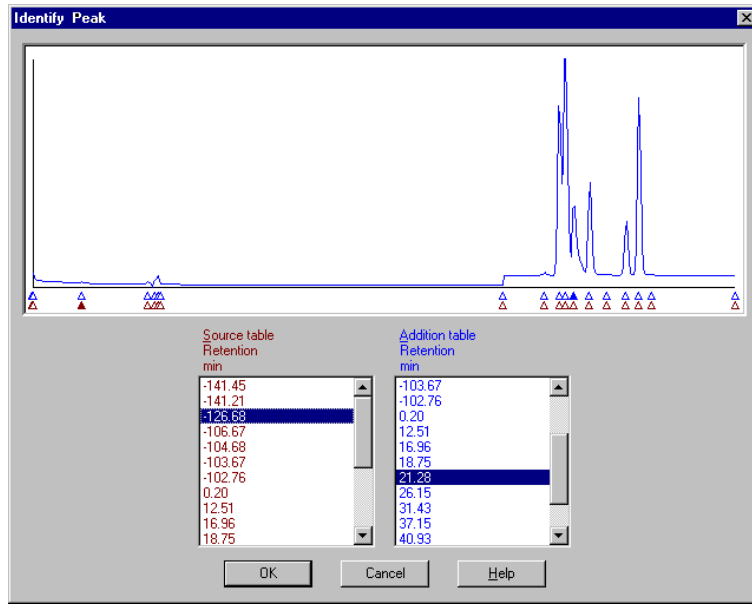


Fig 11-31. Identify Peak dialogue.

8. Locate and select the peak of the unspiked sample. You can select the peak either by clicking its black triangle marker or by highlighting its reference in the **Source table**.

9. Locate the peak for the spiked sample. Select it either by clicking its blue triangle marker or by highlighting its reference in the **Addition table**.

10. Click **OK** to confirm the selection

Evaluating the amount of the component in the sample.

The amount of the component of interest is shown in the peak table of the sample chromatogram.

No	Retention (min)	Area (mAU*min)	Amount (mg)
10	8.42	38.1014	2.165
11	9.59	12.9160	>
12	10.43	28.1310	>
13	11.71	139.5769	>

Fig 11-32. The result shown in the Amount column.

11.4.3 Measuring recovery

For a full explanation of recovery factor measurement, see "Recovery calculation" on page 316.

Preparing for quantitation

An external standard quantitation table for the component of interest must first have been prepared (see "Creating a quantitation table" on page 320). Internal standard quantitation tables cannot be used.

1. Perform a run with the unspiked sample and a run with the spiked sample.

2. Integrate the curves to produce peak tables. During integration, the sample curves must use the same X-axis base unit as the standards. Time is the recommended unit for highest reliability.

3. Check that the integration is correct. If not, consult "Optimising peak integration" on page 262, which describes how to optimise integrations.

4. The peak tables for the unspiked and the spiked sample must be present in the same result file. Open one of the sample result files. Use **File:Open:Peak Tables** to copy the other peak table to that result file.

5. Use **File:Save** to save the peak tables before continuing with the recovery calculations.

Calculating the recovery

1. Select **Quantitate:Calculate Recovery** to open the **Calculate Recovery Factor** dialogue.

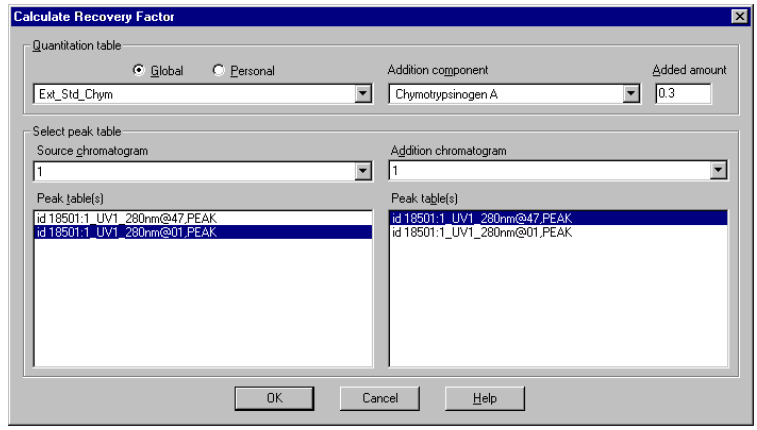


Fig 11-33. Calculate Recovery Factor dialogue.

2. From the **Quantitation table** drop-down list, select the desired quantitation table. If the table was not saved for global use, it is necessary to click the **Personal** button before selecting the table required.

Note: Only external standard quantitation tables will be shown.

3. The peak table for the unspiked sample must now be identified. Open the **Source chromatogram** drop-down list and select the chromatogram which contains the sample peak table. Highlight the peak table in the **Peak table(s)** list.

4. Identify the peak table for the spiked sample. Open the **Addition chromatogram** drop-down list, select the source addition chromatogram and then the peak table in the **Peak table(s)** list.

5. From the **Addition component** drop-down list, highlight the component which was added prior to sample preparation.

6. Enter the injected amount of this component in the **Added amount** field.

7. Click **OK**.

Viewing the results

Using the chromatogram layout detailed above, the recovery factor calculated by the software is located at the bottom of the peak table. You need to scroll to the end of the table to see it.

No	Retention (min)	Area (mAU*min)	Area/Peak area ((time) %)	Height (mAU)
	Recovery Factor ()			0.989
	Component name			Chymotrypsinogen A
	Calculated from			id 18501:1_UV1_280nm
	Baseline			id 18501:1_UV1_280nm@01,BASEC
	Peak rejection on			
	Maximum number of peaks ()			20
	Recovery applied.			
	Current peak filter settings			
	Maximum number of peaks ()			20

Fig 11-34. Recovery factor result.

If the recovery can not be calculated, one of the following signs is shown:

> means that one of the amounts/concentrations is higher than the highest value in the calibration curve i.e. it is outside the valid range of the calibration curve

< means that one of the amounts/concentrations is lower than the lowest value in the calibration curve i.e. it is outside the valid range of the calibration curve

\- means that the recovery factor can not be calculated. For example, this sign might indicate that the peak could not be identified in both runs.

11.5 Automated quantitation

Some method wizards designed for quantitation are available for ÄKTAdesign systems supplied with Autosampler A-900. These can be used to quantitate a sample automatically or to update a quantitation table. These procedures do not work on other systems.

For a general description of procedures, please refer to “Other evaluations” on page 284.

A quantitation table must be produced from standards before samples can be quantitated. The same method must be used for all standard and sample runs.

The Basic10 or Explorer10 strategies contain the Autosampler component option. To add the Autosampler to the strategy, go to System Control and select **Administration:System Setup**. In the resulting **System Setup** dialogue, select either Basic10 or Explorer10 and then click on the **Edit** button. Click on the **Component** button and then check the **AutoSampler** checkbox. This will make the Autosampler A-900 available under the **Injection** dialogue during the Wizard setup.

11.5.1 Creating a quantitation table from standards

1. Start by creating a wizard utilizing the Autosampler A-900. On the dialogue for **Injection** settings, choose AutoSampler.

2. The method and the injection volume must be the same for all the standard runs.On the **Scouting** tab, you may select the correct standard concentration levels by double clicking in the field for the variable **Quantitation_Type** (instruction **QuantitationData** in the text instructions). You can also set the level after the run has been completed. Note that:

- Each level is an alias for a specific concentration of the standard
- Level 1 must be selected for the standard with the highest concentration
- The levels must be set in order of decreasing concentration of the standard
- All runs with the same concentration must be given the same level.

Enter data in the scouting scheme for all the standard runs.

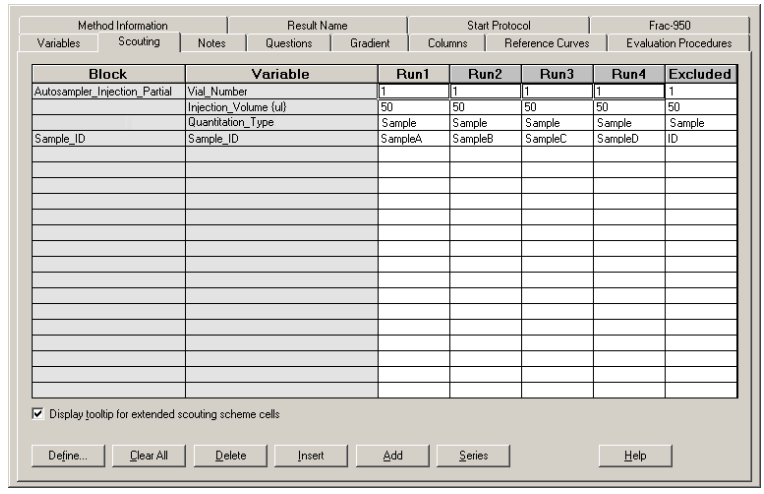


Fig 11-35. Scouting tab used to enter standard data.

3. Open the **Evaluation Procedures** tab and select the procedure **Integrate_and_Print**. This procedure will automatically integrate the first UV curve using default baseline settings.

4. Save the method.

5. Perform all the standard runs.

6. From the Evaluation window, select **Quantitate:Edit Quantitation Table:New** and create a quantitation table manually from the standard runs (see “Creating a quantitation table” on page 320).

11.5.2 Automated Quantitation

1. You must produce a quantitation table in the method editor for the components of interest before samples can be quantitated automatically (see Section above).
2. Use the same method for the sample runs as you used for the standard runs.
3. On the **Scouting** tab, click on **Clear All** to clear the scouting scheme then enter the new values. Double click each **Quantitation_Type** field in turn and select **Sample** for all sample runs.
4. Return to the **Evaluation Procedures** tab and select only the procedure **Quantitate_Sample**. This procedure automatically integrates the first UV curve with default baseline settings and uses the selected quantitation table to quantitate the sample before printing the amounts and concentrations of the components.
5. Click on the **Quantitate** button on the Evaluation Procedures tab and select, from the **Global** or **Personal** folder, the quantitation table to be used. This copies the quantitation table into the **Quantitate_Sample** procedure.

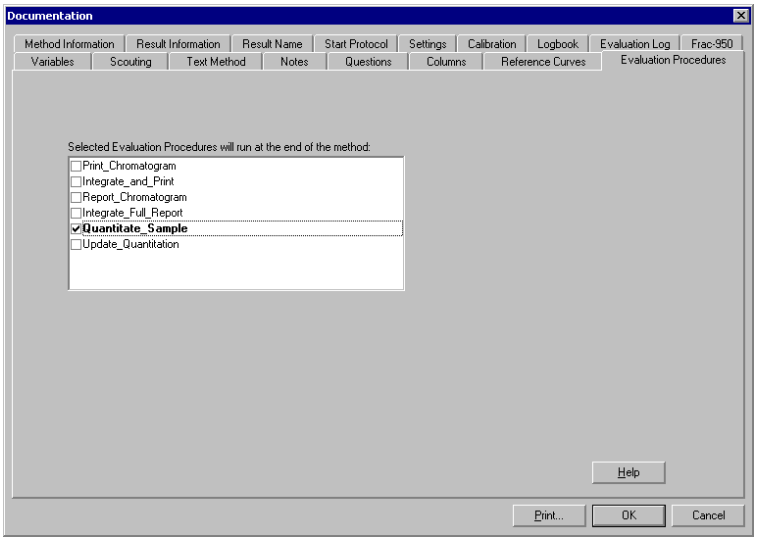


Fig 11-36. Evaluation Procedures tab used to select the procedure.

Note: The procedure can not be executed if a quantitation table has not been selected.

6. Save the method with a new name. Perform the run(s). The amount and concentration of the components in the samples will be printed automatically after each run.

11.5.3 Automated Update

The process of updating a Quantitation table is described in "Updating a quantitation table" on page 335.

A quantitation table for the components of interest must be available before a quantitation table can be automatically updated.

1. Use the same method as was used for the standards when the quantitation table was created.

2. On the **Scouting** tab, click on **Clear All** to clear the scouting scheme then enter the new values. Select the correct concentration level for the standards in the variable **Quantitation_Type**.

3. From the **Evaluation Procedures** tab, select the procedure **Update_Quantitation**. This procedure will automatically integrate the first UV curve using default baseline settings, update the selected quantitation table with the new standard and print an update report.

4. Click on the **Quantify** button on the **Evaluation Procedures** tab and select the quantitation table to be updated. This quantitation table will now be copied into the **Update_Quantitation** procedure.

5. By default, the quantitation table will be updated by **Replace** so that all points at the selected level will be replaced with the new point.

Note: You can only perform one run at each level since the quantitation table will be updated by replacement of the old points.

6. If you want to update by **Average** so that a new average value is calculated from the old points together with the new point, you must edit the procedure. To do this, click **Edit** in the **Evaluation Procedures** tab and the **Procedure Editor** dialogue opens.

   Scroll down the list of instructions in the procedure and select the existing **Update** instruction.

Under **Parameters**, use the scroll bar to locate the **Average or replace** drop-down list. Open the drop-down list and highlight **Average** then click the right hand side **Replace** button to change the instruction.

In the **Procedure editor** dialogue, select **File:Exit**.

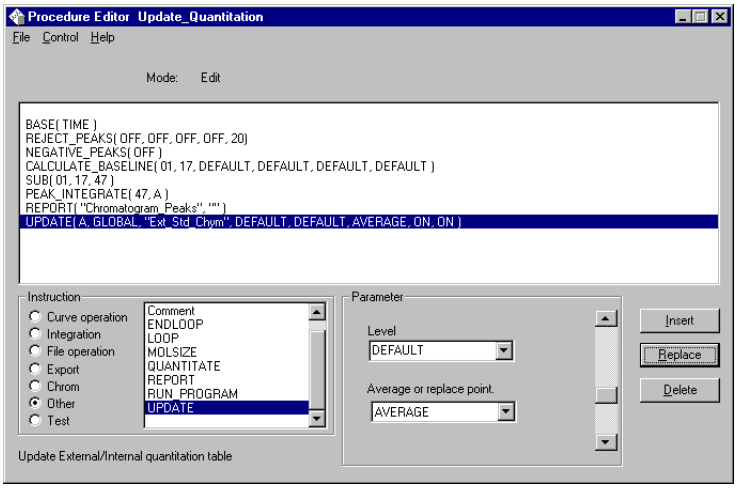


Fig 11-37. Procedure editor dialogue.

7. You can now save the method and perform the runs. The Quantitation table will be updated automatically after each run.

Make sure that the correct concentration level for the standard is selected in the variable **Quantitation_Type** for each run.

11.5.4 Automated update and quantitation in scouting runs

It is possible to run both standards and samples in the same scouting run and to continuously update a previously created quantitation table with new values. To do this, the template and procedures must be slightly modified.

1. You must have a quantitation table available for the components of interest.

2. Open the same method as was used to create the quantitation table from the standard runs.

3. Click on the **Evaluation** tab in **Run Set-up**. Highlight the **Update_Quantitation** procedure from the list and click on the **Quantitate** button. Select the quantitation table to be used and click on **OK**. Deselect the **Update_Quantitation** procedure.

Repeat this for the **Quantitate_Sample** procedure. After this is done, make sure both procedures are de-selected, otherwise they will be run twice.

4. Enter the text instruction mode (select **View:Text instructions**) and select the last instruction in the method (**End_Method**). Select **Other** in the **Instruction** box. Highlight **Evaluate** in the list, open the **Procedure** drop-down list and select a procedure, e.g. **Update_Quantitation**.

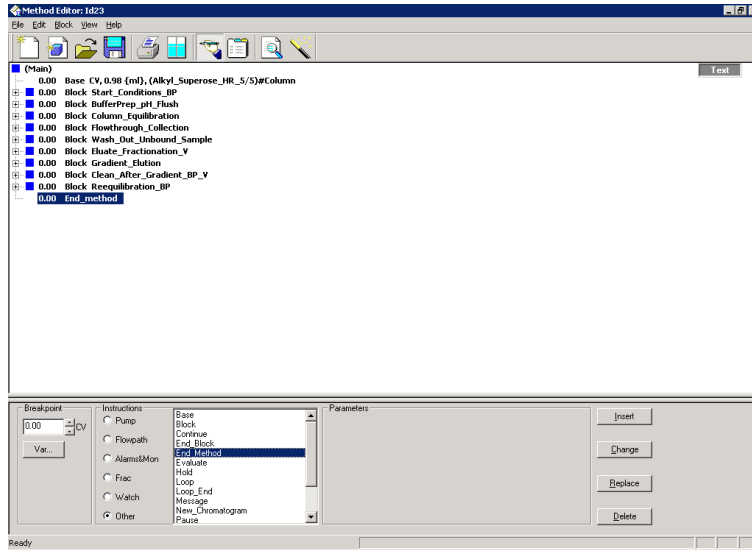


Fig 11-38. Instruction box used to select Evaluation procedures.

Click **VAR** in **Parameters** to open the **Variable name definition** dialogue box. Type a variable name, for example **Procedure** and click on **OK**. The **Evaluate** instruction will be automatically inserted in the method.

Fig 11-39. Evaluate instruction inserted in the method.

By defining evaluation procedure as a variable, different procedures can be selected on the **Scouting** tab for different scouting runs.

5. Select **View:Run Set-up**. Select the **Scouting** tab, click on **Define** and edit the scouting variables list to include **Procedure, Sample_ID**, **Vial_Number**, **Injection_volume** and **Quantitation_Type**.

Note: The **Procedure** variable will appear in the beginning of the list of variables, even though the **Evaluate** instruction is inserted at the end of the method.

6. Set up the scouting scheme. First, all the standards must be run in the scouting scheme. Select the **Update_Quantitation** procedure on the **Scouting** tab for all the standard runs. Make sure that **Quantitation_Type** is set to the correct standard level for each run. The quantitation table will now be updated with new values after each run. Note that you can only perform one run at each level since the quantitation table will be updated by replacement of the old points.

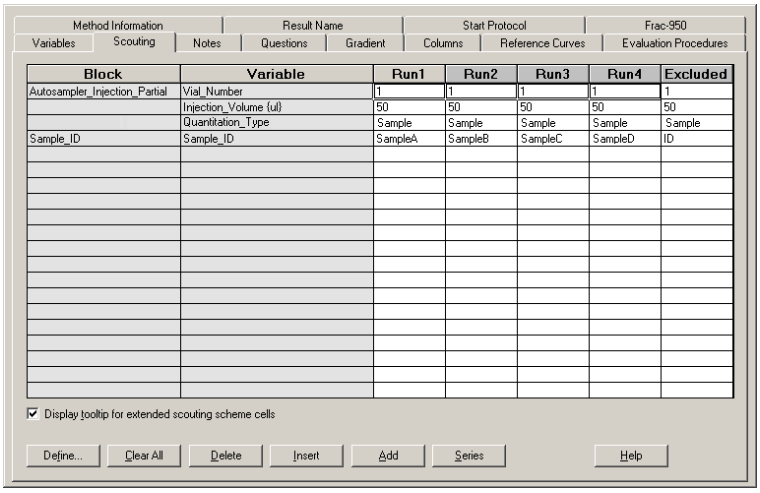


Fig 11-40. The first part of the Scouting tab used to set up the standard runs.

7. If you do not need to perform many runs at each level, skip the following instructions and go on to step 12 below.

8. If you need to perform many runs at each level, the **Update_Quantitation** procedure must be edited to perform the update by average and given a new name. First, a copy of the procedure must be created. Click **Import** in the Evaluation Procedures tab and select the current method used. Select **Update_Quantitation** and give it a new name under **Import as**, for

example **Update_Average**. Click **Import**, then **Close**. This returns you to the Evaluation Procedures tab.

9. Highlight the name of the new procedure and click **Edit** and the **Procedure Editor** opens. Highlight the existing **Update** instruction. Use the **Parameters** scroll bar to locate the **Average or replace point** drop-down list and use it to select **Average**. Click on the **Replace** button to change the instruction. Then select **File:Exit**.

10. Select, in the **Procedure Editor** dialogue, the **Update_Average** procedure, click on the **Quantitate** button and select the quantitation table and click on **OK**. After this is done, de-select all the procedures, otherwise they will be run twice.

11. This new **Update_Average** procedure should then be selected on the scouting page for the second and further runs at each standard level concentration. The **Update_Quantitation** procedure (Update by Replace) should still be used for the first run at each level.

12. After the standard runs have been set up in the scouting scheme, select the samples to be run. Select the **Quantitate_Sample** procedure in the scouting scheme for all the sample runs. Select **Sample** in the variable **Quantitation_Type** for all the sample runs.

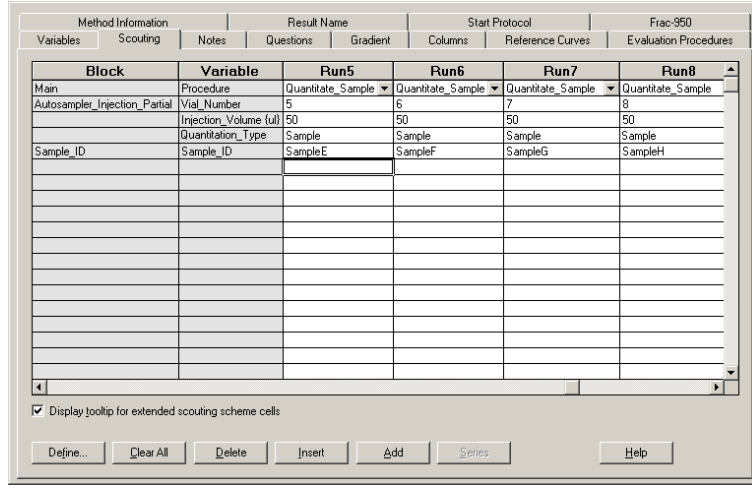


Fig 11-41. The last part of the Scouting page used to set up sample runs.

13. Save the method and perform the runs.

All standards will now be run automatically and the quantitation table will be updated after each run. Then all the samples will be run automatically and the amount and concentration of the components of interest will be printed after each run.

Note: The result files will include an additional chromatogram (labelled 12) containing a small part of the curves collected during the execution of the evaluation procedure.

11.5.5 The evaluation procedure instructions for the Analysis module

Three new procedure instructions are available in the Analysis module. Other procedure instructions available in UNICORN are described in the UNICORN 4.0 Administration and Technical manual.

Instruction	Description	Parameters
QUANTITATE	Calculates the concentration and amounts in the sample from a quantitation table. Amount and concentration columns will be added to the peak table.	Peak table source Global or Personal table Quantitation table name Injection volume in ml*

UPDATE	Updates a Quantitation table with new data from one standard concentration level. The default **Limit (+/-)** value of 12.5% will be used.	Peak table source Global or Personal table Quantitation table name Injection volume in ml* Concentration level for the standard** Average or replace point Save updated table Print updated table
MOLSIZE	Calculates the molecular sizes from a molecular size curve. A Mol. size column will be added to the peak table.	Peak table source Global or Personal table Mol. size table name

*DEFAULT here means that the value will be taken from the Injection volume reported by the Autosampler A-900 from the method. DEFAULT can only be used when the injection is done by the autosampler.

DEFAULT here means that the value will be taken from the level entered in the **QuantitationData instruction in the method.

Before any of these instructions can be executed you must make sure that:

- A suitable Quantitation table is selected. This can be done by entering its name in the Quantitation table name parameter in the instruction. The easiest way to achieve this is to select the appropriate quantitation table in the Method Editor with the **Quantitate** button when the procedure is selected on the **EvalProc** tab.

- A peak table is present in the chromatogram. This can be done by simply including the peak integration instructions prior to the **MOLSIZE**, **QUANTITATE** or **UPDATE** instruction in the procedure.

11.6 Measuring molecular size

The molecular size of components in a sample can be determined by size exclusion chromatography. The column must first be calibrated with components of known molecular size. The retention is inversely related to the molecular size.

The procedure for determining molecular size is described below.

11.6.1 Overview of molecular size determination

There are two stages to the process:

- peak data from a standard are used to produce a molecular size curve
- from the molecular size curve, the molecular size of the components in the sample are determined.

The process is described in detail in the next Section. The following provides an outline of the technique.

Producing the molecular size curve

This process determines the relationship between molecular sizes and corresponding retention values.

1. A run is performed with one or more standards. Please refer to Chapter 6. The standards should contain a number of components of known molecular size and these should extend beyond the limits of size expected in the test sample.

2. Using the Evaluation module, peak integrate the standard curve to produce a peak table. This process is described in Chapter 10.

3. The peak table from the standard is used to produce a molecular size table. Each peak is represented by a retention value. Relevant peaks are selected in turn and data on the corresponding molecular sizes are entered.

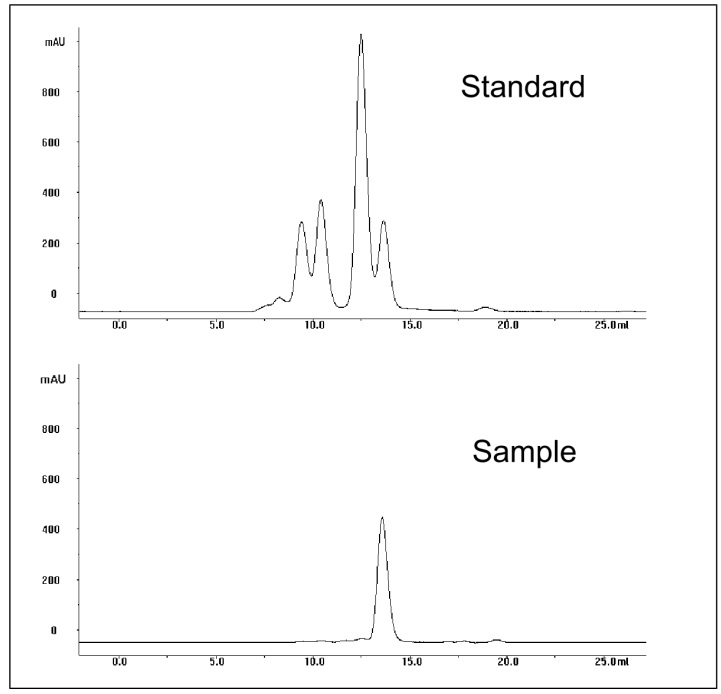


Fig 11-42. Chromatographic curves of the standard and the sample.

4. The software plots these values as a molecular size curve. This shows an inverse relationship between the molecular size and retention.

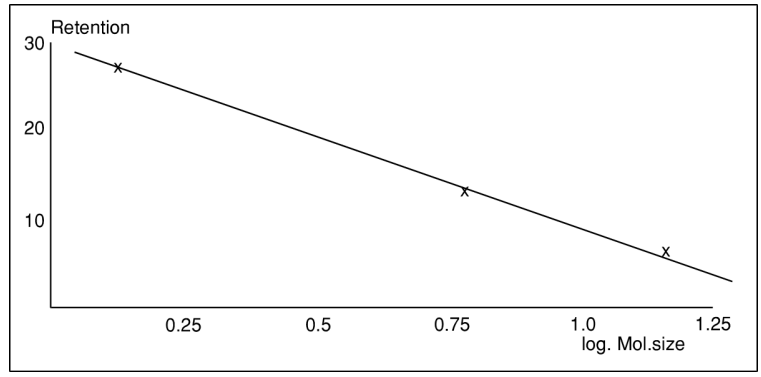


Fig 11-43. Molecular size curve.

Calculating the molecular sizes in the sample

The molecular size table is used to calculate the molecular size of components in the sample.

1. The sample peak table is used to obtain retention values for each of the components of interest.

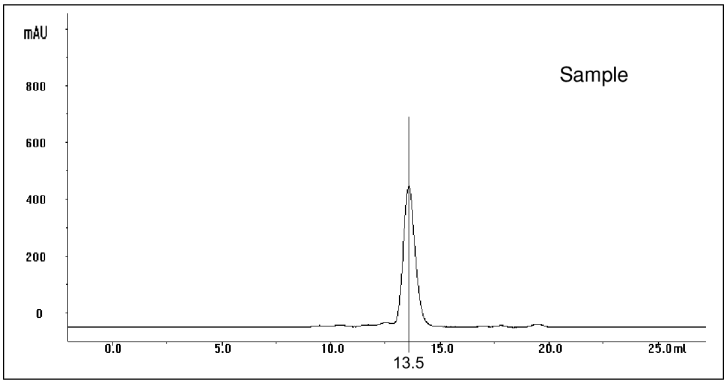


Fig 11-44. Chromatographic curve of the sample.

2. The molecular size curve is used to obtain the molecular sizes of the components in the sample. The molecular sizes are presented in the peak table.

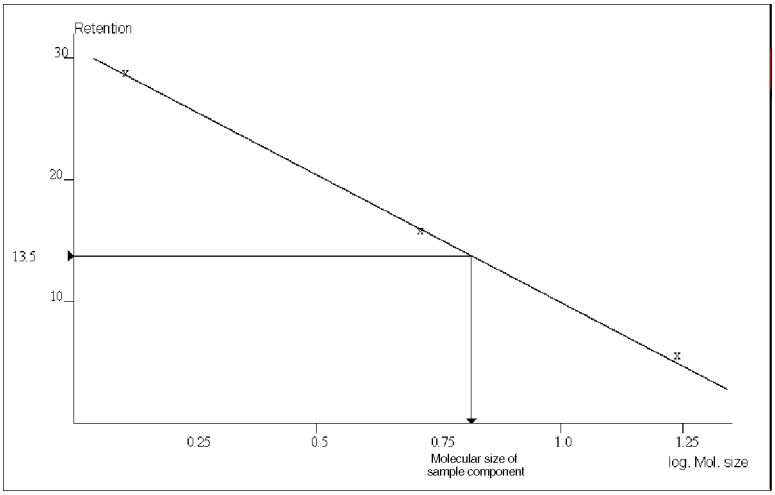


Fig 11-45. Molecular size curve.

11.6.2 Determining molecular size - the process in detail

This Section describes how to:

- produce the molecular size curve
- calculate the molecular sizes of the components in the sample

Producing the molecular size curve

Before creating the molecular size curve, you need to perform chromatographic runs with an appropriate standard with components of known molecular size. The standard should contain components of size which extend over the range expected in the sample. If you are using many components, it may be better to split them into two or more standard runs.

After the chromatographic runs, the curves must be peak integrated to produce the peak tables. During integration, the standard curves must all use the same X-axis base unit. Volume is the recommended unit for molecular size determination.

Use **File:Save** to save the peak table before continuing.

Selecting the standard peak tables

1. From the Evaluation module, select **Mol. Size:Edit Mol. Size Table:New**. The **New Molecular Size Table** dialogue opens.

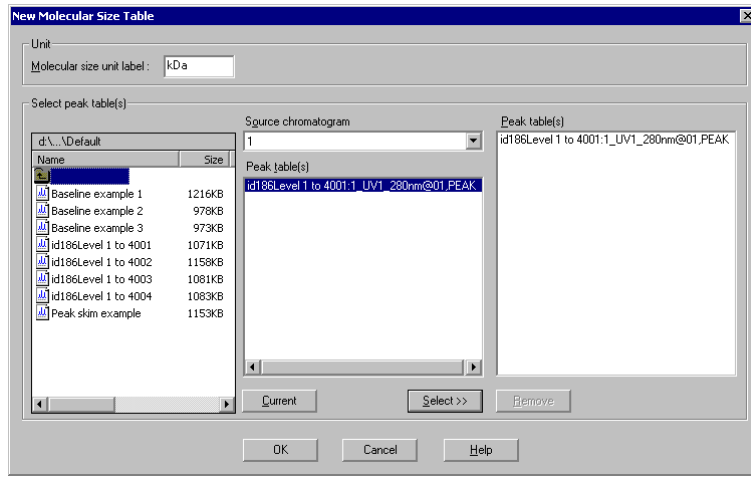


Fig 11-46. New Molecular Size Table dialogue.

2. Select a result file by double clicking its icon.

3. Open the **Source chromatogram** drop-down list and highlight the source chromatogram required. Clicking **Current** at any time returns you to the chromatogram which was active before entering **Mol. Size**.

4. Highlight a peak table which was prepared from the standard and click **Select** (or double click the peak table) to transfer it to the right hand side **Peak table(s)** list.

5. You can select further peak tables, providing all the runs are made under the same conditions.

6. If you need to de-select a table, highlight it in the right hand window then click on **Remove**.

7. Peak tables from other chromatograms can be added to the peak tables list. To do this, select a result file as before, highlight the source chromatogram and select the peak table(s) required.

8. Once the **Peak table(s)** list contains all the items required, click **OK** to open the **Molecular size table** dialogue.

9. If the unit of size measurement is different from the default kDa, enter the appropriate unit in the **Molecular size unit label** field.

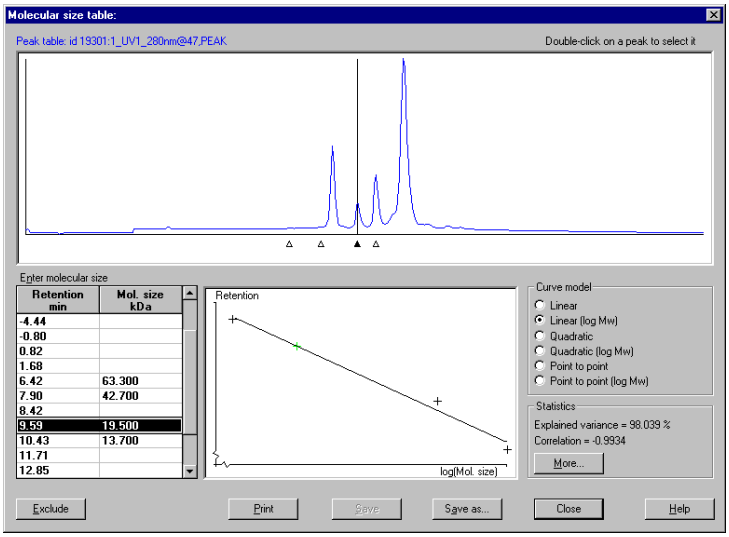


Fig 11-47. Molecular size table dialogue.

You use this dialogue to select the peaks which will be used to produce the molecular size curve. Each curve and its peak table name is colour coded. All the available peaks for all curves are listed together in the **Retention/Mol.size** table.

Selecting the peaks and entering molecular size data

Select the components of known molecular size (by double clicking) and enter their molecular sizes in the table. The triangles show the peaks that have been selected.

When a peak is selected, the name of its source peak table is shown above the curve window. The text is coloured to correspond with the related curve. This is useful when you wish to know which of two closely spaced peaks, from different peak tables, has been selected.

Using data on the standard, enter the molecular size for each of these peaks as follows:

1. Select the first valid peak by double clicking it on the curve or in the table.

2. Double click in the **Mol. size** column and enter the known molecular size from the standard.

3. Repeat steps 1 and 2 for all the components of known molecular size.

4. If a mistake is made, a peak can be excluded by highlighting its entry in the table and clicking **Exclude** or by double clicking it again.

Choosing the molecular size curve model

The molecular size curve shows the relationship between molecular size and the corresponding retention. The curve is plotted from the Retention/Mol.size data that you have entered in the table. Before this can be done, a curve model is needed which describes the relationship between molecular size and retention.

1. Choose the appropriate **Curve model** by clicking its button from the options available:

    - Linear
    - Linear (logMw) (theoretically, this is the best choice)
    - Quadratic
    - Quadratic (logMw)
    - Point to point
    - Point to point (log Mw)

Each of the peaks selected is represented by a point in this curve which is drawn according to the best fit that can be achieved using the model selected.

Try different models until a satisfactory fit is found (See Statistics below).

2. You can select a peak by:

   clicking it in the curve

   clicking its entry in the **Retention/Mol. size** table

   clicking its plotted point in the molecular size curve.

   Whatever method is used, the peak or peak reference will be highlighted in all three views.

Statistics

Apart from the two point to point models, the molecular size curves can be expressed as a mathematical expression. This expression and related items can be viewed by clicking on **Statistics**.

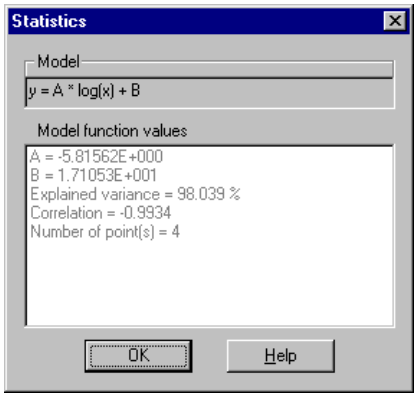


Fig 11-48. Statistics dialogue.

The expression, shown at the top of the window, is followed by the values for the constants it contains.

Correlation, which is only displayed for linear models, should be as close as possible to -1.00.

The explained variance value should be as close as possible to 100%, but is usually rather high even for poor models. For instance, a value of 90% indicates a very poor model. The best model may be selected

by testing different models and comparing the explained variance values, but you still need to examine the molecular size curve visually to ensure a good fit.

Please refer to Appendix C for mathematical and statistical details.

Saving and printing the molecular size table

1. Click **Save as** to open the **Save molecular size table** dialogue, which shows a listing of existing molecular size tables.

Fig 11-49. Save molecular size table dialogue.

2. Enter the new name into the **Molecular size table name** window. You can choose whether the table will be globally accessible to any user or only to someone using your user id. The default is **Global**. Select **Personal** if you need to restrict access.

3. Click **OK** to save the table. Once the table is saved under **Save as**, it can be updated using the same file name by use of the **Save** button. Care is needed as this overwrites the original table. You might prefer to use **Save as** and a new name after any editing operations and so preserve the original molecular size table.

4. In the **Molecular size table** dialogue, click **Print** to obtain a print-out of the molecular size curve, statistics and molecular size table.

5. Click **Exit** to conclude the molecular size process.

To print a molecular size table that was prepared previously:

1. Open the molecular size table (see "Opening, Renaming or Deleting a molecular size table" on page 365).

2. Click **Print** to print the data.

Opening, Renaming or Deleting a molecular size table

To open a previously saved molecular size table:

1. Select **Mol. Size:Edit Mol. Size Table:Open**.

2. If you wish to access tables available only to your user id, click **Personal**.

3. Highlight the name of the table you require in the **Molecular size table(s)** list to copy it to the **Molecular size table name** field.

4. Click **OK** to open the table or **Cancel** to abort the operation.

To rename a molecular size table:

1. Select **Mol. Size:Edit Mol. Size Table:Rename**.

2. If you wish to rename a table available only to your user id, click **Personal**.

3. Highlight the name of the table you wish to rename in the **Molecular size table(s)** list.

4. Type the new name for the table in the **Molecular size table name** field.

5. Click **OK** to rename the selected table or **Exit** to abort the rename procedure.

To delete a molecular size table:

1. Select **Mol. Size:Edit Mol. Size Table:Delete**.

2. If you wish to delete a table available only to your user id, click **Personal**.

3. Using the **Molecular size table(s)** list, highlight the table to be deleted.

4. Click **Delete** to delete the table or **Exit** to abort the delete procedure.

Calculating the molecular size of components in a sample

The molecular size curve defines the relationship between retention values and molecular size. This curve can now be used to determine the molecular sizes of components in the sample.

Preparing for molecular size calculations

1. Perform a run with the sample.

2. Open the sample result file and peak integrate the curve to produce a peak table. When integrating, the sample curve must use the same X-axis base unit as the standards. Volume is recommended for molecular size calculations.

3. Use **File:Save** to save the peak table before continuing with the molecular size calculations.

Selecting the molecular size table

1. Select **Mol. Size:Calculate Mol. Size**.

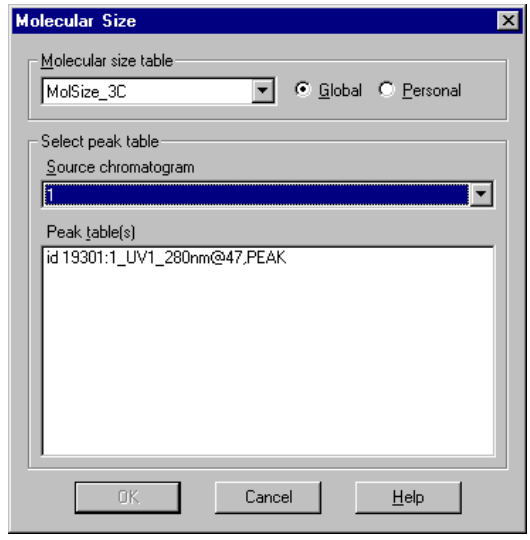


Fig 11-50. Molecular Size dialogue.

You use the molecular size dialogue to choose the molecular size table you are going to use and the sample peak table to which it is applied.

2. Select **Global** or **Personal** according to the location of the molecular size table.

3. Open the **Molecular size table** drop-down list and select the table required.

4. To select the sample's peak table open the **Source chromatogram** drop-down list and select from the displayed list. The related peak tables are then shown in the **Peak table(s)** list. Highlight the table required.

5. Click **OK** to calculate the molecular sizes.

Displaying the results

The results of the molecular size determination are shown in the Mol. size column of the peak table.

No	Retention (min)	Mol. size (kDa)
8	6.42	>
9	7.90	38.22
10	8.42	31.15
11	9.59	19.57
12	10.43	14.05
13	11.71	<

Fig 11-51. Display of results.

If the molecular size cannot be calculated, one of the following signs is shown in the **Mol. size** column:

> means that the molecular size is larger than the largest size in the molecular size curve, i.e. it is outside of the valid range.

< means that the molecular size is smaller than the smallest size in the molecular size curve, i.e. it is outside of the valid range.

\- means that the retention value is negative

When the result file is saved, it includes the molecular size table that was used for the molecular size determination. You can later view the table that was used by selecting **Mol. Size:Edit Mol. Size Table:View Current**.

If you want to print the table that was used, select **File:Report** and select the **Quantify and Mol. Size** option (see "Modifying an existing report format" on page 250).

A Evaluation functions and instructions

This appendix describes the functions implemented in the evaluation module. There are four sections in the appendix:

A.1 describes how the smoothing functions are calculated

A.2 gives an basic introduction into baseline calculation theory which is an essential part of peak integration

A.3 describes the peak table column components

A.4 the Procedure Editor instruction types are described which are used to build up an evaluation procedure

A.1 Smoothing algorithms

A.1.1 Moving Average

For each data point in the source curve, the processed curve is calculated as the average of the data points within a window centred on the source data point. The width of the window is determined by the parameter value, expressed as number of data points.
When the source point is less than half the window size from the beginning or the end of the curve, the average is calculated symmetrically round the source point over as many data points as possible.
Increasing the window width increases the smoothing effect.
The filter algorithm only accepts odd integer parameter values between 1 and 151. If an even number has been given it is incremented by one.

A.1.2 Autoregressive

The first data point in the source curve is copied to the processed curve. For each subsequent data point, the previous processed point is multiplied with the parameter value and added to the current source data point. The result is then divided by the parameter value plus 1 according to the following formulae:

$$t_1 = S_1$$

$$t_n = \frac{(p * t_{n-1} + S_n)}{(p + 1)}$$

where:

t_n = current processed point
t_{n-1} = previous processed point
S_n = current source point
p = smoothing parameter value

Increasing the parameter value increases the smoothing effect. The filter algorithm accepts integer parameter values between 1 and 25.

A.1.3 Median

For each data point in the source curve, the processed curve is calculated as the median of the data points within a window centred on the source data point. The width of the window is determined by the parameter value, expressed as number of data points.
When the source point is less than half the window size from the beginning or the end of the curve, the median is calculated symmetrically round the source point over as many data points as possible.
Increasing the window width increases the smoothing effect. To completely remove a noise spike, the window width should in principle be slightly more than twice the width of the spike.
The filter algorithm only accepts odd integer parameter values between 1 and 151. If an even number has been given it is incremented by one.

A.1.4 Savitzky-Golay

The Savitzky-Golay algorithm is based on performing a least squares linear regression fit of a polynomial of degree k over at least k+1 data points around each point in the spectrum to smooth the data. The derivative is then the derivative of the fitted polynomial at each point. The calculation uses a matrix formalism to calculate 1st through 9th derivatives. The calculation is performed with the data in low X to high X order. If the input trace goes from low to high, it is reversed for the calculation and then re-reversed afterwards.

For more information on the Savitzky-Golay algorithm, see Steiner et al., 1972, Analytical Chemistry, 44:1906.

A.2 Baseline calculation theory

The baseline calculation can schematically be described in three steps:

1. Defining baseline segments
2. Select baseline points
3. Draw the baseline.

A.2.1 Defining baseline segments

In the first step, baseline parameters are used to find the baseline segments. The parameters can be seen in the **Integrate:Calculate baseline** function or by pressing the **Baseline settings** button in the **Integrate:Peak integrate** function. The default values for the parameters are determined from the source curve.
The baseline segments are found by different parameters based on the type of algorithm selected.

Morphological algorithm

The **Morphological** algorithm searches for all parts of the source curve which:

1. come into contact at both extremes of the **Structure width**.

   This parameter is based on the widest detected peak in the curve.

2. fulfils the **Minimum distance between data points**.

   This parameter reduces the total number of data points created from a curve.

Classic algorithm

The **Classic** algorithm searches for all parts of the source curve which:

1. are longer than the **Shortest baseline segment**.

   This parameter determines the minimum length for a part of the source curve to be considered a possible baseline segment.

2. have no point outside the **Noise window**.

   The noise window is defined as a rectangular corridor parallel to the slope of the curve and centred on the first and last points within the currently inspected segment.

3. slope less than the **Slope limit**.

This limits the maximum slope of the baseline to differentiate baseline segments from peaks.

4. are lower than the **Max baseline level**.

   Determines the highest acceptable signal level for the baseline. This parameter is by default set to have no influence on the baseline calculation and is seldom necessary to adjust.

The parameters can be illustrated as a rectangular box in which the source curve has to fit to be identified as a baseline segment (Fig A-1 on page 372). The length of the box corresponds to the **Shortest baseline segment** and the height of the box corresponds to the maximum level of noise on the baseline segments and is referred to as the **Noise window**.

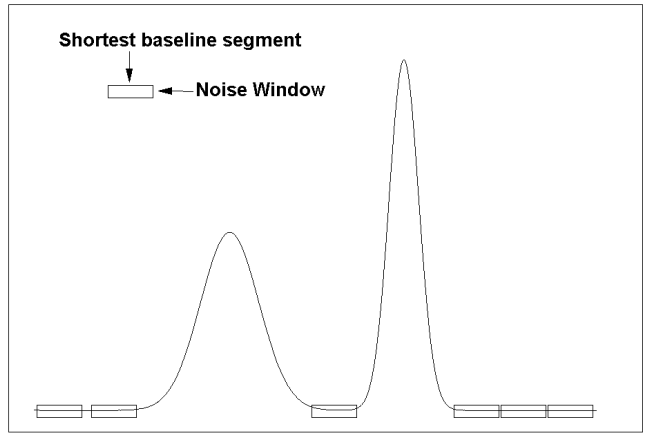


Fig A-1. Baseline box with Shortest baseline segment and Noise window.

The rectangular box is allowed to be tilted with a maximum slope corresponding to the **Slope limit** (see Fig A-2 on page 373). The box is not allowed to move up above the **Max baseline level**.

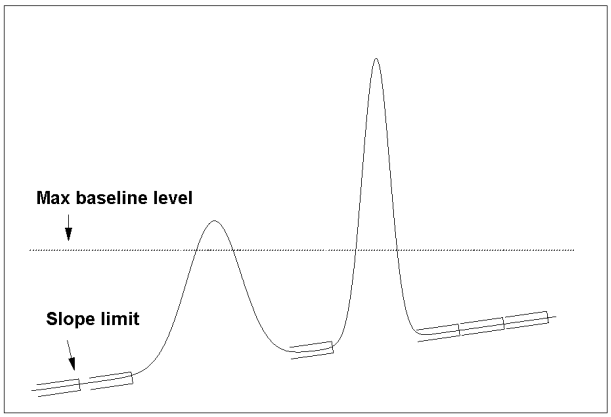


Fig A-2. Slope limit and Max baseline level.

When looking for baseline segments, the box is virtually moved along the source curve in steps of 1/3 of the **Shortest baseline segment.** A baseline segment is found whenever the currently examined part of the source curve fits completely within the box. The found baseline segments are joined by connecting adjacent segments, provided that the slope of the joining lines does not exceed the **Slope limit**.

A.2.2 Selecting baseline points (for Classic algorithm)

In the second step, the baseline segments are replaced by a large number of baseline points. A baseline point is placed at the start and end of each segment. The line between these will also be filled with points. The baseline points are shown as pale blue crosses in the **Integrate:Edit baseline** function (see "Manually editing a baseline" on page 276).

A.2.3 Drawing the baseline

The baseline points are used to create the baseline curve using a spline interpolation. The spline function ensures that the baseline curve is guided by the baseline points, but the curve does not necessarily pass through them. The baseline will thus be a smoothly curved function passing close to or through the points. To reduce the effect of noise on the peak integration, the created baseline is adjusted by forcing it equal to the source curve in every position where the difference between the baseline and the source curve is small enough. If the **Accept negative peaks** option (see "Including negative peaks" on page 266) is off, the baseline will be forced down to the level of the source curve whenever the created baseline goes above the source curve.

A.2.4 Estimating the baseline parameters from the source curve (for Classic algorithm)

The baseline parameters can sometimes be difficult to set. Rough estimates can be found by analysing the source curve.

Measuring the Shortest baseline segment using curve co-ordinates

If you are uncertain of the length of the **Shortest baseline segment**, you can try to measure it directly on your chromatogram. Locate the shortest segment of the curve that you consider as a part of the baseline and measure the length of the segment using the marker box on the chromatogram (see "Optimising the baseline parameters using a classic algorithm" on page 269). Insert this value as the **Shortest baseline segment** value.

Measuring the noise level using curve co-ordinates

As for measuring the Shortest baseline segment, curve co-ordinates can be used in exactly the same manner to measure noise levels on the source curve. First use the Zoom function to select a part of the curve representative of the baseline noise. Measure the Y-axis co-ordinates, using the appropriately selected Y-axis scale, to calculate the noise range as the difference between the max. and min values. Add an extra 20% and insert this value as the **Noise window** value.

A.2.5 Measuring the Slope limit using Differentiate and curve co-ordinates (for Classic algorithm)

To measure the slope at any point on the curve:

1. Select **Operations:Differentiate**. A dialogue will appear.

2. Select the desired source curve, check that a **First order calculation** is selected and click on **OK**. The differentiated curve will appear in the active chromatogram.

3. Measure the Y-axis values on the differentiated curve using the **Marker** curve co-ordinates function. Remember to select the appropriate Y-axis scale. Any Y-axis value is interpreted as the UV curve slope at the selected retention point.

   If the differentiated curve is very noisy, it can be filtered using a light Moving average filter in the **Operations:Smooth** function (see "Smoothing a curve" on page 200).

4. Determine the highest slope value of the baseline (non-peak) part of the curve. Add 10% and insert this value as the **Slope limit**.

A.3 Peak table column components

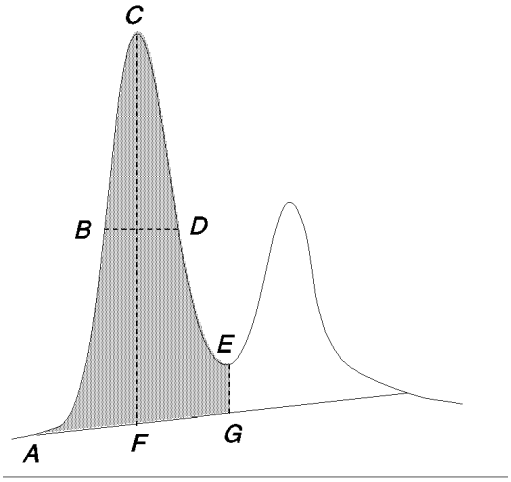


Fig A-3. Diagram illustrating peak parameters. See the parameter list below for explanations.

Column name	*Description*
Peak name	Name of peak.
Retention	(time or volume base) Retention at the peak maximum (C in Figure A-3).
Width	(time or volume base) Difference in retention between the peak end and peak start (G-A in Figure A-3).
Area	(time or volume base) Calculated as the area between the curve and baseline, between the peak start and peak end (shaded in Figure A-3).
Height	Maximum amplitude above the baseline (C-F in Figure A-3).
Peak endpoint retention	(time or volume base) Retention value at peak start and peak end (A, G in Figure A-3).

Width at half height	(time or volume base) Calculated by taking the maximum height of the peak above the baseline, then determining the peak width at half this value above baseline (D-B in Figure A-3, where BD bisects CF).
Percent of total area	(time or volume base) Peak area as a percent of the total area under the curve above the baseline. Note that this value may differ in time and volume base if the flow rate is not constant throughout the method.
Percent of total peak area	(time or volume base) Peak area as a percent of the sum of all integrated peaks. Note that this value may differ in time and volume base if the flow rate is not constant throughout the method.
Type of peak limits	Identifies the criteria for peak start and peak end as either the baseline intersection or dropline to the baseline.
Peak endpoint heights	Amplitude above the baseline at left (A in Figure A-3) and right peak limits (E-G in Figure A-3).
Fraction tube id	Fraction number at peak start, peak maximum and peak end.
Baseline height	Baseline amplitude at peak start, peak maximum and peak end (A, F and G in Figure A-3).
Sigma	Standard deviation for a Gaussian-shaped peak. For definition see below*
Resolution	Peak resolution. For definition, see “Measuring resolution” on page 283 and below**.
Capacity factor	For a definition, see below ***. The Capacity factor will only be calculated when the chromatogram is in volume base. The total liquid volume, Vt, must be entered in the **Integrate** dialogue for this parameter to be calculated.

Kav	Gel phase distribution constant in gel filtration. For definition, see below ****. Kav will only be calculated when a gel filtration column was used and when the chromatogram is in volume base. The void volume, V0, must be entered in the **Integrate** dialogue for this parameter to be calculated.
Plate height (HETP)	Height equivalent to theoretical plate and plates/metre. The column height must be entered in the Integrate dialogue for this parameter to be calculated. For definition, see "Measuring HETP" on page 282 and below‡.
Asymmetry	Peak asymmetry (indicator of column packing). For definition, see "Measuring peak asymmetry" on page 283 and below§.
Concentration	Values calculated by the Analysis module (only available if the Quantitation module is installed).
Amount	As above
Molecular size	As above

***Sigma**

$$\text{Sigma} = \sqrt{\frac{\frac{(x_n - x_1)}{n - 1}\sum_{i=1}^{n}\left(y_i\,(x_i - x_{ymax})^2\right)}{A_{peak}}}$$

where:
n is the number of data points

x is the volume or time value

y is the amplitude value

x_{ymax} is the volume or time value at the maximum amplitude value

A_{peak} is the area of the peak
The peak width for a Gaussian peak is (4 x Sigma).

****Resolution**

$$R = \frac{(V_{R2} - V_{R1}) \times 1.177}{w_{h1} + w_{h2}}$$

where:

V_{R1} = retention volume for peak 1
V_{R2} = retention volume for peak 2
w_{h1} = peak width at half height for peak 1 (for Gaussian peaks)
w_{h2} = peak width at half height for peak 2 (for Gaussian peaks)

The peak resolution is calculated with one of the following three algorithms:

1) $(V_{R2} - V_{R1})/((W_{b2} + W_{b1})/2)$
2) $(V_{R2} - V_{R1})/((Sigma_2 + Sigma_1) \times 2)$
3) $(V_{R2} - V_{R1})/(2 \times (W_{h2} + W_{h1})/2.354$

where V_{R1}, W_{b1}, $Sigma_1$ and W_{h1} are the retention, width, sigma and width at half height of the previous peak, and V_{R2}, W_{b2}, $Sigma_2$ and W_{h2} are the retention, width, sigma, and width at half height of the current peak, respectively. The UNICORN.INI variable (EVAL) ResolutionAlg determines which of the three algorithms is actually used. If this variable has the value 1, 2, or 3, then the algorithm used corresponds to the numbered list above. If the variable has the value 0, or if the variable is not defined or has a value other than 0, 1, 2, or 3, then the default (3) algorithm is used.

To change the peak resolution algorithm, edit the UNICORN.INI file by:

1. Minimize UNICORN and locate the file UNICORN.INI within C:\UNICORN\BIN.
2. Open the file and locate the following line:

   ```
   EVAL ResolutionAlg
   ```

   If the line does not exist then add it before the "`Begin`" line.
3. Choose the desired value for the algorithm.
4. Save the file.

Note: Do not make any changes in the UNICORN.INI file between the lines "`Begin`" and "`End`" as this may severely affect the functionality of UNICORN.

*** **Capacity factor**

$$k' = \frac{V_R - V_t}{V_t}$$

where:

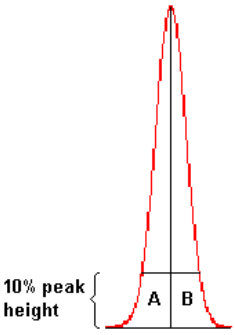
V_R = retention volume
V_t = total liquid volume

**** **K_{av}**

$$k_{av} = \frac{V_R - V_0}{V_C - V_0}$$

where:

V_R =retention volume
V_0 = void volume
V_C = column volume



§**Asymmetry**

Asymmetry = width B / width A, where A and B are the partial peak widths measured at 10% of the peak height, with A representing the leading part of the peak and B the tailing part of the peak.

‡ **HETP**

HETP = L/N

$N = 5.54 \times (V_R/w_h)^2$

where:

N = no. of theoretical plates
L = bed height in cm
V_R = peak retention volume or time
w_h = peak width at half height expressed in the same units as V_R
Plates/meter is the number of theoretical plates per meter, N x (100/L)

A.4 Evaluation procedure

A.4.1 Curve operations

Instruction	*Description*	*Parameters*
ADD	Adds two curves to gain a third curve which is the sum of the two curves. The two source curves must have the same Y-axis unit and not be fraction or injection curves or else a run time error will occur.	First source curve Second source curve Target curve position
AMP_MUL	Multiplies the amplitude of the source curve by the multiplication factor and stores the result in the target curve position.	Source curve Target curve position Multiplication factor
AMP_SHIFT	Shifts the amplitude of the source curve by the shift factor and stores the result in the target curve position.	Source curve Target curve position Shift factor
CLEAR	Clears specified curve from the working memory of the computer.	Source curve
COPY	Copies the source curve to target curve position.	Source curve Target curve position
CUT	Cuts out the part of the source curve between Left and Right Limits and stores the result in the target curve position.	Source curve Target curve position Left limit Right-limit
DERIVATE	Differentiates the source curve (first or second order) and stores the result in target curve position. The Y-axis of the target curve position will be a normalised scale without unit.	Source curve Target curve position First Order or Second Order

DIV	Divides two curves to gain a third curve which is the quotient of the two curves. The two source curves can have any Y-axis unit. The Y-axis of the target curve position will be a normalised scale without unit.	First source curve Second source curve Target curve position
HISTO-GRAM	Creates a histogram from any non-fraction curve (source curve 1) and a fraction curve (source curve 2_frac), and stores the result in the target curve position. If source curve 2 is not a fraction curve a run time error will occur. The Y-axis of the target curve position will be the same as that of the first source curve.	First source curve Second source curve Target curve position
INTEGRATE	Performs a mathematical integration of the source curve and stores the result in Result curve. This instruction is not the same as Peak integrate which performs a real peak integration.	Source curve Target curve position
POOL_ FRACTIONS	Pools fractions from the source curve and stores the result in the target curve position. The fractions are pooled from the first selected fraction to the last selected fraction. If source curve is not a fraction curve, or First or Last is not an existing identification, a run time error will occur.	Source curve Target curve position First fraction to pool Last fraction to pool
RET_MUL	Multiplies the retention of the source curve by the Multiplication factor and stores the result in the target curve position.	Source curve Target curve position Multiplication factor

RET_SHIFT	Shifts the retention of the source curve by the Shift factor and stores the result in the target curve position.	Source curve Target curve position Shift factor
SIMULATE_ PEAK-FRAC	Simulates Peak Fractionation.	Source curve Target curve position Frac size Width Start slope End slope
SMOOTH_ AR	Smooths source curve with an autoregressive filter and stores the result in target curve position. The Filter parameter decides the strength of the filter.	Source curve Target curve position Filter
SMOOTH_ MA	Smooths the source curve with a moving average filter and stores the result in Resulting Curve. The Filter width parameter decides how many samples wide the filter is.	Source curve Target curve position Filter width
SMOOTH_ MEDIAN	Smooths the source curve with a median filter and stores the result in target curve position. The Filter width parameter decides how many samples wide the filter is.	Source curve Target curve position Filter width
SMOOTH_ SG	Smooths curve with Savitzky-Golay algorithm.	Source curve Target curve position Filter width Polynomial order
SUB	Subtracts two curves to gain a third curve which is the difference of the two curves. The two source curves must have the same Y-axis unit and not be fraction or injection curves.	First source curve Second source curve Target curve position

TDIV	Divides two curves to gain a third curve which is the quotient of the two curves. The two source curves can have any Y-axis unit. The threshold values are used to avoid division of numbers close to zero. At those points where source curve 1 has amplitude less than Threshold1, or source curve 2 has amplitude less than Threshold2, the result of the division is defined to be 1.0. The Y-axis of the curve will be the same as that of the first source curve.	First source curve Second source curve Target curve position Threshold1 Threshold2

A.4.2 Integration

Instruction	*Description*	*Parameters*
CALCULATE_BASELINE	Calculates a baseline from the source curve. The baseline is stored in the target curve position. DEFAULT can be selected in the Baseline parameters which will then calculate default baseline parameters for each new curve.	Source curve Target curve position Noise Window Shortest baseline segment Slope limit Max baseline level
CALCULATE_BASELINE_MORPH	Calculates a baseline from the curve crvSrc using a morphological method. The baseline is stored in curve crvDst.	Source curve Destination curve Noise Window Width Distance Between Points
CLEAR_PEAKTABLE	Clears the peak table in Peak table source from the working memory of the computer.	Peak table source

COPY_ PEAKTABLE	Copies a peak table from Peak table source to Resulting peak table.	Peak table source Resulting peak table
NEGATIVE_ PEAKS	Controls the baseline behaviour in subsequent baseline calculations. If OnOff is ON then the baseline may be drawn above the curve and negative peaks may be detected by PEAK_INTEGRATE. If OnOff is OFF then the baseline is never drawn above the curve.	OnOff
PEAK_ INTEGRATE	Performs a peak integration on the source curve and stores the resulting peak table in Resulting peak table. It is assumed that the baseline is subtracted.	Source curve Resulting peak table
PEAK_ WINDOW	Specifies which part of the source curve that will be integrated. Peaks between retention Left limit and Right limit will be detected if the OnOff parameter is set to On. If OnOff is set to Off, the whole curve will be used for integration.	Source curve Left limit Right limit OnOff
REJECT_ PEAKS	Any combination of conditions is allowed. If all parameters are OFF then every detected peak are included in the peak table.	Height less than Width less than Width more than Area less than Peak must be one of xx largest
SET_ COLUMN_ HEIGHT	Sets the column height for the peak integration calculation of the HETP value. The Column height parameter is the height of the column in centimetres. If Column height is OFF then the HETP value is not calculated for the following integrations.	Column Height

SET_ COLUMN_V 0	Sets the void volume for peak integration calculation of Kav.	Void volume
SET_ COLUMN_V T	Sets the total liquid volume for peak integration calculation of the capacity factor.	Total liquid volume
SET_ SKIM_SIZE_ RATIO	Sets the Skim size ratio to be used in the following peak integration(s)	Ratio

A.4.3 File Operations

CURVE_ OPEN	Opens the curve specified in the Result file defined in File name and stores it in target curve position. If "*" is entered as File name the current result file will be used. The File name parameter may include a path from the users root folder.	File name Curve name Target curve position
IMPORT_ CURVE	Imports a curve to the current chromatogram from another chromatogram (in the current file) and stores it in the target curve position.	Chromatogram name source curve Target curve position
IMPORT_ PEAKTABLE	Imports a peak table to the current chromatogram from another chromatogram (in the current file) and stores it in the target curve position.	Chromatogram name Peak table source Resulting peak table
PEAKTABLE _OPEN	Opens the specified Peak table in the Result file defined in File name and stores it in the Resulting peak table. If "*" is entered as File name the current Result file will be used. The File name parameter may include a path from the current users root folder.	File name Peak table name Resulting peak table

A.4.4 Export

Instruction	*Description*	*Parameters*
EXPORT_ CURVE_AIA	Exports curve in AIA format	Source curve Export to file
EXPORT_ CURVE_ ASCII	Exports the Source curve to the file defined in Export to File in ASCII format. In the part of source curve limited by Left limit and Right limit Every <n> samples are exported.	Source curve Left limit Right limit Every <n> sample Export to file
EXPORT_ CURVE_WKS	Exports the source curve to the file defined in Export to File in WKS format. In the part of source curve limited by Left limit and Right limit Every <n> samples are exported.	Source curve Left limit Right limit Every <n> sample Export to file
EXPORT_ EVAL_LOG_ ASCII	Exports an evaluation log in ASCII format to the file defined in Export to file.	Export to file
EXPORT_ EVAL_LOG_ WKS	Exports an evaluation log in WKS format to the file defined in Export to file.	Export to file
EXPORT_ EVAL_LOG_ XLS	Exports an evaluation log as XLS format to the file defined in Export to file.	Export to file
EXPORT_ METHOD_ ASCII	Exports a method to the file defined in Export to file in ASCII format. If all parameters are OFF then no method is exported. If Main is ON then the main method is included and if Blocks is ON then all blocks are included in the exported file.	Main Blocks Export to file

EXPORT_ METHOD_ WKS	Exports a method to the file defined in Export to file in WKS format. If all parameters are OFF then no method is exported. If Main is ON then the main method is included and if Blocks is ON then all blocks are included in the exported file.	Main Blocks Export to file
EXPORT_ METHOD_ XLS	Exports a method to the file defined in Export to file in XLS format. If all parameters are OFF then no method is exported. If Main is ON then the main method is included and if Blocks is ON then all blocks are included in the exported file.	Main Blocks Export to file
EXPORT_ MULTI_ CURVES_ ASCII	Exports multiple curves (previously defined with EXPORT_SEL_CURVES instructions) in ASCII format to the file defined in Export to file.	Export to file
EXPORT_ MULTI_ CURVES_WKS	Exports multiple curves (previously defined with EXPORT_SEL_CURVES instructions) in WKS format to the file defined in Export to file.	Export to file
EXPORT_ MULTI_ CURVES_XLS	Exports multiple curves (previously defined with EXPORT_SEL_CURVES instructions) in XLS format to the file defined in Export to file.	Export to file
EXPORT_ NORMALISE_ RETENTION	Normalises retention when exporting multiple curves.	None
EXPORT_ PEAKTABLE_ ASCII	Exports the peak table in Peak table source to the file defined in Export to file in ASCII format.	Peak table source Export to file

EXPORT_ PEAKTABLE_ WKS	Exports the peak table in Peak table source to the file defined in Export to file in WKS format.	Peak table source Export to file
EXPORT_ PEAKTABLE_ XLS	Exports the peak table in Peak table source to the file defined in Export to file in XLS format.	Peak table source Export to file
EXPORT_ PEAKTABLE_ XML	Exports the peak table in XML format.	Peak table source Export to file
EXPORT_SEL _ CURVES	Selects a curve for subsequent export (using the EXPORT_MULTI-CURVES_* instruction). The curve is cut according to the right and left cut limit and the number of points to be exported may be set by the Export every (for example, fifth point) parameter.	Source curve Left cut limit Right cut limit Export every

EXPORT_ DOC_400_ ASCII	Exports the documentation in the current result file in ASCII format to the file defined in Export to file. If all parameters to this function are OFF then no documentation is exported. If at least one of them is ON then the documentation will be exported and the corresponding parts will be included in the exported file.	ONOFF Variables ONOFF Scouting ONOFF Start Protocol ONOFF Questions ONOFF Ref-Curves ONOFF Eval-Proc ONOFF Method Info ONOFF Method Notes ONOFF Start-Notes ONOFF Run-Notes ONOFF Eval-Notes ONOFF Sys Settings ONOFF Calibration ONOFF Log-Book ONOFF Result Name ONOFF Column Parameters NAME ONOFF Result Info ONOFF Method Sign ONOFF Result Sign Export to file

EXPORT_ DOC_400_ WKS	Exports the documentation in the current result file in WKS format to the file defined in Export to file. If all parameters to this function are OFF then no documentation is exported. If at least one of them is ON then the documentation will be exported and the corresponding parts will be included in the exported file.	ONOFF Variables ONOFF Scouting ONOFF Start Protocol ONOFF Questions ONOFF Ref-Curves ONOFF Eval-Proc ONOFF Method Info ONOFF Method Notes ONOFF Start-Notes ONOFF Run-Notes ONOFF Eval-Notes ONOFF Sys Settings ONOFF Calibra tion ONOFF Log-Book ONOFF Result Name ONOFF Column Parameters NAME ONOFF Result Info ONOFF Method Sign ONOFF Result Sign Export to file

EXPORT_ DOC_400_ XML	Exports the documentation in the current result file in XML format to the file defined in Export to file. If all parameters to this function are OFF then no documentation is exported. If at least one of them is ON then the documentation will be exported and the corresponding parts will be included in the exported file.	ONOFF Variables ONOFF Scouting ONOFF Start Protocol ONOFF Questions ONOFF Ref-Curves ONOFF Eval-Proc ONOFF Method Info ONOFF Method Notes ONOFF Start-Notes ONOFF Run-Notes ONOFF Eval-Notes ONOFF Sys Settings ONOFF Calibra tion ONOFF Log-Book ONOFF Result Name ONOFF Column Parameters NAME ONOFF Result Info ONOFF Method Sign ONOFF Result Sign Export to file

EXPORT_ DOC_WKS	Exports the documentation in the current result file in WKS format to the file defined in Export to file. If all parameters to this function are OFF then no documentation is exported. If at least one of them is ON then the documentation will be exported and the corresponding parts will be included in the exported file.	ONOFF Variables ONOFF Scouting ONOFF Start Protocol ONOFF Questions ONOFF Ref-Curves ONOFF Eval-Proc ONOFF Method Info ONOFF Method Notes ONOFF Start-Notes ONOFF Run-Notes ONOFF Eval-Notes ONOFF Sys Settings ONOFF Calibra tion ONOFF Log-Book ONOFF Result Name ONOFF Column Parameters NAME Export to file

EXPORT_ DOC_XLS	Exports the documentation in the current result file in XLS format to the file defined in Export to file. If all parameters to this function are OFF then no documentation is exported. If at least one of them is ON then the documentation will be exported and the corresponding parts will be included in the exported file.	ONOFF Variables ONOFF Scouting ONOFF Start Protocol ONOFF Questions ONOFF RefCurves ONOFF EvalProc ONOFF Method Info ONOFF Method Notes ONOFF StartNotes ONOFF RunNotes ONOFF EvalNotes ONOFF Sys Settings ONOFF Calibration ONOFF LogBook ONOFF Result Name ONOFF Column Info ONOFF BufferPrep NAME Export to file

EXPORT_ DOC_ASCII	Exports the documentation in the current result file in ASCII format to the file defined in Export to file. If all parameters to this function are OFF then no documentation is exported. If at least one of them is ON then the documentation will be exported and the corresponding parts will be included in the exported file.	ONOFF Variables ONOFF Scouting ONOFF Start Protocol ONOFF Questions ONOFF Ref-Curves ONOFF Eval-Proc ONOFF Method Info ONOFF Method Notes ONOFF Start-Notes ONOFF Run-Notes ONOFF Eval-Notes ONOFF Sys Settings ONOFF Calibration ONOFF Log-Book ONOFF Result Name ONOFF Column Parameters NAME Export to file

A.4.5 Chromatogram functions

Instruction	*Description*	*Parameters*
COPY_ CHROM	Creates a copy of the specified chromatogram. If "*" is used as source then the current (default) chromatogram is used. If "*" is used as destination then a default name will be created for the copy.	From chromatogram name To chromatogram name
CREATE_ NEW_ CHROM	Creates a new chromatogram with the given name. If "*" is used for the chromatogram name a default name will be generated and used.	Name
DELETE_ CHROM	Deletes the named chromatogram. If trying to delete the current (default) chromatogram a run time error will be caused.	Chromatogram name
OPEN_ CHROM	Opens the specified chromatogram from the specified file.	File name Chromatogram name
RENAME_ CHROM	Renames the specified chromatogram. If "*" is used as From then the current (default) chromatogram is used.	From chromatogram name To chromatogram name
RESTORE_ DESTINATION _CHROM	Resets the destination for the subsequent curve and peak table operations to the default chromatogram. Used in pair with the SET_DESTINATION_CHROM instruction.	
SET_ DESTINATION _CHROM	Opens the named chromatogram as destination for the subsequent curve and peak operations. Used in pair with the RESTORE_DESTINATION _CHROM instruction.	Chromatogram name

A.4.6 Other

BASE	Sets the X-axis base in which the following calculations will be done in. If the value of X-axis base is DEFAULT then the default base is used (usually the base the method was run in). This instruction should be the first in the evaluation procedure otherwise it will have no effect at all.	X-axis base
Comment	Inserts a comment below the marked instruction	Comment text
ENDLOOP	Marks the end of a LOOP statement.	
LOOP	The instructions between this statement and the ENDLOOP statement are repeated n times. It is possible to have loops within loops as long as the number of LOOP statements matches the number of ENDLOOP statements.	n Number of loops
MOLSIZE	Calculates the molecular sizes from a molecular size curve. A Mol. size column will be added to the peak table.	Peak table source Global or Personal table Mol. size table name
QUANTITATE	Calculates the concentration and amounts in the sample from a quantitation table. Amount and concentration columns will be added to the peak table.	Peak table source Global or Personal table Quantitation table name Injection volume in ml*

REPORT	Prints a report with the specified named report layout and title. If Title is "*" then the title in the report layout is used. If Report-Layout is "*" then a default lay-out is used.	Report layout Report title
RUN_ PROGRAM	Starts a program as a separate process. The Program name string contains the program name and parameters to start it with.	Program name
UPDATE	Updates a Quantitation table with new data from one standard concentration level. The default **Limit (+/-)** value of 12.5% will be used.	Peak table source Global or Personal table Quantitation table name Injection volume in ml* Concentration level for the standard** Average or replace point Save updated table Print updated table

B Troubleshooting

B.1 Logon problems

B.1.1 Unable to log on to UNICORN

Choose your username from the list and enter your password. If you have forgotten your password, ask the system administrator for a new one.
If you cannot log on using your correct username and password, the USERS30.MPM file in the \UNICORN\SERVER\FIL folder may be corrupt. Restore the file from the latest back-up copy or reinstall the default user (see the UNICORN 4.0 Administration and Technical manual).
If users are not available on a remote station in a network installation (the user list in the **Logon** dialogue box is empty), make sure that the computer is logged on to the network before starting UNICORN. A remote station accesses the user list directly from the network server. If the user list on a local station in a network installation is not up to date, make sure that the computer is logged on to the network before starting UNICORN. The user list is stored locally on a local station, and is updated automatically from the network server if the computer is logged on to the network.

B.1.2 Error message "Strategy file error

If you receive the error message "Strategy file error. Can't load strategy" in a stand-alone installation, the strategy file is probably corrupt. Reinstall the strategy as described in the UNICORN 4.0 Administration and Technical manual. In a network installation, the error may appear if you try to create a method for a system not physically connected to the computer. Make sure that the computer is logged on to the network before UNICORN is started so that the strategy file on the server disk is accessible.

B.2 UNICORN access problems

Note: When experiencing UNICORN access problems, it will often be necessary to make reference to the UNICORN 4.0 Administration and Technical manual.

B.2.1 Unable to access certain UNICORN functions

UNICORN functions to which you do not have access appear grey in the menu and cannot be used. Your user profile is determined by the system administrator from **Administration:User Setup** in the main menu.

B.2.2 Connections are not available

Check the connection between the PC and the chromatography system. Check that the power to the chromatography system is turned on. If the connection appears to be correct and the power is turned on, switch off the chromatography system and quit UNICORN. Shut down and switch off the computer, then restart the entire system.
If a system is not available when you attempt to establish a connection, check that you have access rights to the system. Access rights are not automatically assigned for a newly defined system.
If you receive the error message "Cannot connect to system ..." in a network installation, check:

- that the local computer to which the system is connected is turned on and logged on to the network.
- that the computer from which you are trying to establish a connection is logged on to the network.
- that the limit of 8 connections to the system has not been exceeded.

If you can establish a connection but cannot control the system (the manual menu commands in system control are grey), check that no other user has a control mode connection, and that you have sufficient access rights to control the system manually.

B.2.3 Run data Connection in System Control displays a “No x”

In System Control, if the Run data option **Connection** says “No 1” or “No 2”:

- Check that the UNICORN PC Control board is configured according to the settings made during the installation of the program, i.e. the same Control unit number, Address and IRQ must be set at the Control board.
- The communication may also fail if the UNICORN PC Control board configurations conflict with other boards in the PC. If this is the case select a free Address and a free IRQ during UNICORN installation and at the Control Board.

If the Run data option **Connection** says “No 3” there is no contact with the OCI:

- In Main Menu select **Administration:System Setup**. In the dialogue select the system with problems and click on Edit. Check that the strategy, pipe server name and the control number are correct according to installation at the local station physically connected to the system.

- If connecting to a system remotely check that the local station (physically connected to the system) is turned on and that the network is functioning at both the remote and the local station.
- Check that the limit of 8 connections to the system has not been exceeded.
- Try restarting the system.

B.3 Method and run problems

Cannot Quit or Logoff from UNICORN

If you are unable to Quit or Logoff from UNICORN for a connection, you may be running a scouting method or a MethodQueue. These functions require a control mode connection in order to start subsequent cycles correctly.

Monitor signals do not appear in the system control Curves panel

For monitor signals to be displayed in system control, they must be set to STORE ON in system settings.
Signals for which STORE ON is set can be chosen from the **View:Curve contents** dialogue box for display in the curves panel.

Error message "Couldn't create result file

If you receive an error message "Couldn't create result file... Destination path could not be found" at the end of a method, the local computer was unable to access the folder specified in the result file path. This may arise if the specified folder is on the network server and network communication has been lost. The result file is saved in the FAILED folder on the local station.

The Method-System Connection dialogue keeps appearing

If the **Method-System Connection** dialogue keeps appearing you have some method(s) that you have not connected to a system, most likely from imported methods using the **Copy from external** function in the Main Menu (see “Copy files from external” on page 27). Connect the method(s) to the appropriate system and the dialogue disappears.

The method editor window does not fit on the screen

If the Method Editor window does not fit on the screen and has scroll bars, you may have the incorrect font size installed. For pre-installed Windows NT 4.0 the display screen resolution is set at “1024x768x65536” with “Large fonts”. You need to install the “Small fonts”, which requires that you have available the Windows NT Workstation 4.0 CD-ROM shipped with your Compaq computer. Insert the CD-ROM and follow the directions on the screen.

Note: Be sure to always install the latest NT4 service pack after installing something from the Windows NT 4.0 CD-ROM.

There are red instructions in a method

Red instructions, i.e. instructions with a red dot, in a method are syntax errors and may be due to the following:

- The method was connected to a wrong system, i.e. the strategy of the system is incompatible with the method.
- The method instructions do not correspond to the components you have chosen for your system. Check your system components under **Administration:System Setup:Components** in Main Menu.
- The **Copy** function was used instead of **Copy from external** when importing a method from a diskette.
- The wrong system may have been selected in the **Save As** dialogue in Method Editor.
- You may also have templates not intended for your system, which often happens for custom designed systems.

There are several actions that you can take:

- Check that the method has been connected to the correct system, either in the **System Method Connection** dialogue when using the **Copy from external** dialogue or in the **Save As** dialogue in Method Editor.
- For custom designed systems go to the Method Editor, select the red instruction and either delete it or replace it with a corresponding instruction, if available, from the Instruction box. Repeat this for all red instructions before saving the method.

I've logged out of Windows NT and then logged in again but I can not get system connection in UNICORN (only for local systems, not remote)

If you shut down Windows NT using the command **Start:Shutdown: Close all programs and log in as a different user**, you will not be able to obtain a system control connection in UNICORN the next time you or another user logs on. This is because the aforementioned shutdown procedure automatically shuts down a number of processes, including those needed for system connection, that are only started when the computer is booted up. In other words, you must restart the computer in order to obtain a system connection in UNICORN.

Print screen does not send a copy of the screen to the printer

Print screen only makes a copy of the screen to the clipboard and not to the default printer. If you wish to make a print out of the view on the screen, press the <Print Scrn> key and paste the image from the clipboard into an appropriate program, such as Microsoft®Paint, and then print out the image.

B.4 Evaluation problems

B.4.1 Incorrect date and time

The date and time recorded in the result file are taken from the PC system clock setting. If these are not correct, check the system clock setting.

B.4.2 Evaluation procedure aborts

Instructions in an evaluation procedure address curves by identification number irrespective of curve names. Make sure that the curves processed when the procedure is executed are compatible with those processed when it was recorded. An evaluation procedure aborts if you try to store resulting curves at the position of an original raw data curve.

B.5 ÄKTAdesign system specific problems

B.5.1 Connected to a system but no system contact

In System Control, if the Run data option **Connection** says "Yes", but the option **Instruments** says "Scanning" and there is no contact with the system after a period of waiting, check if:

- The chromatography system is turned on
- All cable connections are intact

B.5.2 Flowscheme

If the flowscheme is not working properly check that you have selected 65536 colours under Windows NT Start:Settings:Control Panel: Display:Settings.

C Mathematical models and statistics

The Analysis Module (see Chapter 11) uses data from standard runs to produce calibration curves for use over a range of sample concentrations. The accuracy of the interpolation that this provides depends on the quality of the curve fit model employed.
This appendix describes the models that are available. It also describes the Analysis Module's statistical tools which you use to measure how well the model fits the data from the standard(s).

C.1 The curve fit models used by the Analysis Module

The Analysis Module provides a comprehensive range of curve fit models. For the production of calibration curves, these are:

- linear
- linear through origin
- quadratic
- quadratic through origin
- point to point.

Note that it is the average peak size for all points at a specific level that is used for calculating the calibration curve.
The following curve fit models are available for molecular size curves:

- linear
- linear (log Mw)
- quadratic
- quadratic (log Mw)
- point to point
- point to point (log Mw).

For all but the point to point models, the Analysis Module provides values for the appropriate constants used in each curve equation. It also provides statistical data that you can use to assess the quality of fit of the curve to the data.

C.2 How the curve fit models are determined

In the following descriptions, the text to the left describes the model used for the curve fit and the mathematics used by the Analysis Module to evaluate the constants. The screen image to the right is typical for the model described and you obtain it by clicking the Statistics button in the Quantitation table/Mol. size table dialogue boxes. You can find further information about explained variance and correlation in Section D3.

Linear
Based on the equation:

$$y = Ax + B$$

The constants A and B are determined by linear least squares regression. (Please see a statistics textbook for further information.)
A variant of this model is available for use in the production of a molecular size curve. This uses the logarithm of the molecular size as the x value in the above expression.

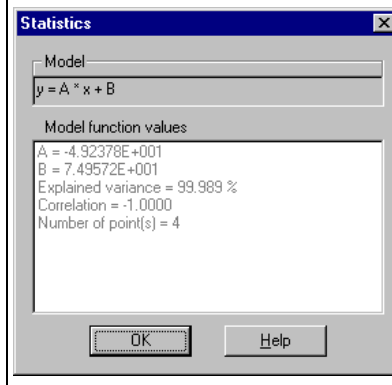


Fig 3-1. Statistics table for the linear model.

Minimum no. of points required:	2 (at least 4 recommended)
Measuring range for the calibration curve:	within the highest and lowest values for the points.

Linear through the origin

Based on the equation:

$$y = Ax$$

The constant A is determined by linear least squares regression. (Please see a statistics textbook for further information.)

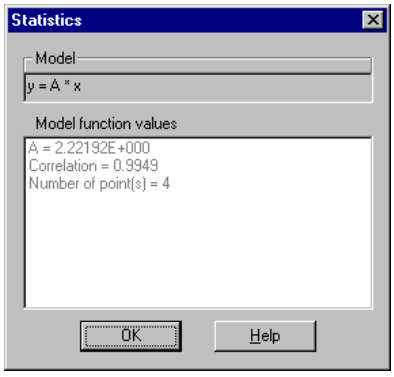


Fig 3-2. Statistics table for the linear through origin model.

Minimum no. of points required: 1 (at least 2 recommended)

Measuring range for the calibration curve: from the point with the highest value down to the origin.

Quadratic

Based on the equation:

$$y = Ax^2 + Bx + C$$

The constants A, B and C are determined by linear least squares regression. (Please see a statistics textbook for further information.)
A variant of this model is available for use in the production of a molecular size curve. This uses the logarithm of the molecular size as the x value in the above expressions.

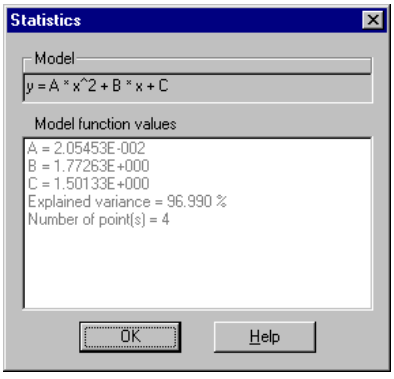


Fig 3-3. Statistics table for the quadratic model.

Minimum no. of points required: 3 (at least 6 recommended)

Measuring range for the calibration curve: within the highest and lowest values for the points.

Quadratic through origin
Based on the equation:

$$y = Ax^2 + Bx$$

The constants A and B are determined by linear least squares regression. (Please see a statistics textbook for further information.)

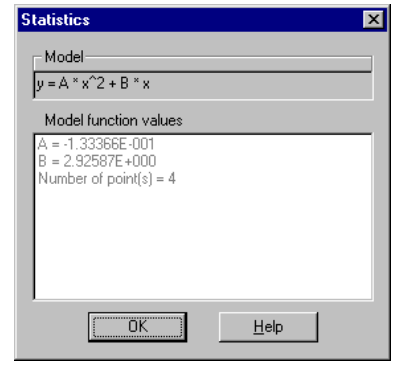


Fig 3-4. Statistics table for the quadratic through origin model.

Minimum no. of points required: 2 (at least 4 recommended)

Measuring range for the calibration curve: from the point with the highest value down to the origin.

Point to point
As these are not based on a single equation, no statistical data is available. The statistics table contains only information on the number of points in the curve.

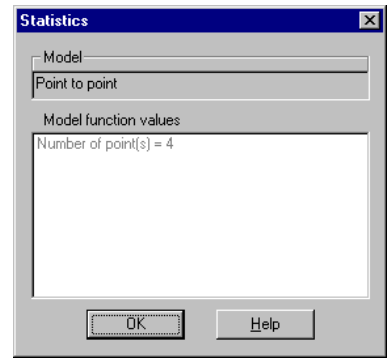


Fig 3-5. Typical statistics table for a point to point model.

Minimum no. of points required: 2

Measuring range for the calibration curve: within the highest and lowest values for the points.

C.3 The statistics available

C.3.1 Correlation

For linear models, the Analysis Module calculates the correlation coefficient which shows how well the data are linearly related. The correlation is displayed in the Statistics table.
If you are producing a calibration curve relating peak area or height to amount or concentration, you aim to achieve a high positive correlation coefficient. A value of +1 indicates a perfect fit of all the data to the straight line.

Note: If you have only two data points for a "Linear" model, or only one point for a "Linear through origin" model, the fitted straight line will inevitably pass exactly through the points. By definition, this leads to a correlation of exactly +1 but this does not indicate a good fit but instead too few data points. In these cases the Statistics table will show a "---" symbol instead of the correlation value.

A molecular size curve has a negative slope so the aim is towards a correlation coefficient of -1 (with the same note regarding too few points).

Correlation is derived as follows:

$$Correlation = \frac{\sum_i [(x_i - \bar{x})(y_i - \bar{y})]}{\sqrt{\left[\sum_i (x_i - \bar{x})^2\right]\left[\sum_i (y_i - \bar{y})^2\right]}}$$

where $\bar{x}$ and $\bar{y}$ are the averages of the x and y values respectively. For the molecular size model "Linear log(Mw)", $\bar{x}$ is the average of the logarithms of the molecular sizes.

C.3.2 Explained variance

Explained variance provides a measure of how much of the variation in the data points (xy pairs) is explained by the model. The remaining variation can be attributed to noise, i.e. random errors, or selection of an inappropriate model. This makes it possible to use the explained variance value for model selection, e.g. to decide if a quadratic model fits the data better than a linear one (indicated by a higher explained variance value). The explained variance is not calculated for curve models drawn through the origin.

The explained variance value is equal to R^2 adjusted for degrees of freedom (see a statistical textbook for further information).

$$\text{Explained variance (\%)} = 100 \times \left[1 - \frac{SS_{residuals}/(n-k-1)}{SS_{total}/(n-1)} \right]$$

where

$$SS_{residuals} = \sum_{i=1}^{n} (y_i - \hat{y}_i)^2 \quad \text{(Residual Sum of Squares)}$$

$$SS_{total} = \sum_{i=1}^{n} (y_i - \bar{y})^2 \quad \text{(Total Sum of Squares)}$$

$\bar{y}$ = Average of all y values

$\hat{y}_i$ = Function value using the fitted model.

For example, $\hat{y}_i = Ax_i^2 + Bx_i + C$

n = Number of points (xy pairs)

k = Number of x terms in the model

For example, 1 for “linear” and 2 for “quadratic”.

Note: You can only obtain a value for explained variance if you have sufficient data points on the curve. For instance, if you only have two points for a “Linear” model, or only three points for a “Quadratic” model, the fitted curve will pass exactly through the points. By definition, this leads to an undefined value for explained variance. In these cases the Statistics table will show a “---” symbol instead of the explained variance value.

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